ISOTOPIC DETERMINATION OF REGION OF ORIGIN IN MODERN PEOPLES: APPLICATIONS FOR IDENTIFICATION OF U.S. WAR-DEAD FROM THE VIETNAM CONFLICT

By

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To Ronald D. Reed, Ph.D., Brigadier General, USAF
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It is with bittersweet appreciation that I thank an incredible mentor for taking a risk and agreeing to this absolutely insane adventure; for the opportunities he provided and his unwavering confidence in me. It is with great regret that I cannot share my success in this endeavor with him. I was fortunate to know him and I dedicate this work to his memory.

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The views expressed in this article are those of the author and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the U.S. Government.

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By

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August 2006

Chair: Anthony B. Falsetti
Major Department: Anthropology

This study is novel in that it is the first of its kind to compile a reference sample of isotopic values associated with known natal regions to be utilized in forensic work. Stable isotopes of carbon, oxygen, strontium, and lead were examined to determine if natal origins could be assessed isotopically between Southeast Asian and American dental remains as well as regionally within the United States. Teeth believed to be of East Asian origin were compared to the extracted third molars of recent American dental patients. Living subjects completed surveys detailing physiological, behavioral, and residential information that affect isotope values. The least squares means for all isotope values examined exhibited significant differences between the East Asian and American cohorts. Based on this information, a discriminant function was created that correctly classified individuals, through resubstitution and cross-validation, as belonging to one of these two groups by 95% or better. The sexes differed significantly as to their carbon ratios with females displaying more enriched values than males. Significant differences

were also noted for $\delta^{13}C$ means among those who have never used tobacco products and those who partook of smokeless tobacco. American strontium values displayed a distinct trend toward homogenization, with the mean value for $^{87}Sr/^{86}Sr$ varying only slightly from that of seawater. In order to identify natal origin among Americans, nine regions were created within the United States based on $\delta^{18}O$ values. Good discrimination was noted between the mountain states and the southern states. A discriminant function analysis proved disappointing though, and additional sampling from most states is needed to improve the statistical robusticity of the model. The results of this study will have wide-reaching effects across the medico-legal spectrum. This body of research will serve as the foundation for a database of modern, human, geolocational isotope values that will assist not only in the identification of fallen servicemen and women, but in the identification of victims of mass fatality incidents, undocumented aliens who perish attempting entry into the U.S., and local skeletal “Jane and John Doe” cases.

CHAPTER 1
STABLE ISOTOPES

Ascertaining the national origin of unidentified human remains is problematic, especially with the passage of time. Often, the number of identifiable bony elements is so few, fragmentary and/or degraded by the chemical properties of the soil, that estimating biological profiles and DNA analyses cannot effectively be performed. This challenge is particularly acute for the Joint POW/MIA Accounting Command's Central Identification Laboratory (JPAC-CIL). The identification of unknown remains believed to be missing U.S. service personnel is frequently hampered by high levels of degradation and fragmentation as a result of circumstances of loss and subsequent taphonomic regimes. If the geo-political region of origin for a set of remains could be established, it would facilitate the construction of identification shortlists, especially from large, open-ended decedent populations. This, in turn, would provide a highly effective means of excluding possible candidates for identification, notably for human remains whose provenience is either unknown or suspect. One potential tool in determining geolocational origins of skeletal material is that of stable isotope analyses. Developed primarily in the geochemical community (Fogel et al. 1997), stable isotope work has revolutionized the anthropological realm, beginning with pioneering, archaeological, dietary studies in the late 1970s (DeNiro & Epstein 1978a and 1978b, van der Merwe & Vogel 1978).

Isotopes of a particular element are atoms whose nuclei contain the same number of protons but differ in their number of neutrons (Hoefs 2004). It is the number of protons

in the atom that determines what the element is as well as how many electrons the atom has (Herz & Garrison 1998). An atom at rest has a neutral charge; therefore, the normal state for an atom is to have the same number of protons within the nucleus as electrons outside of the nucleus. As stated previously, isotopes vary because of the differing number of neutrons within the nucleus. This neutron variation, will in turn, affect the atomic masses of different isotopes of the same element because the atomic mass is a measure of the sum of the number of protons and neutrons (Hoefs 2004).

For example, carbon has an atomic number of “6,” meaning an atom of carbon contains 6 protons within the nucleus. Even though the number of protons is constant within a carbon atom, it can take on three isotopic forms: ^{12}C, ^{13}C, ^{14}C. A carbon atom with a mass of 12 (denoted ^{12}C) has 6 protons and 6 neutrons, one less neutron than a carbon atom with a mass of 13 (^{13}C) and two fewer neutrons than ^{14}C.

Since chemical reactions are largely determined by the ionic or atomic electron configuration, the varying isotopes of an individual element will have the same chemical properties (Schwarcz & Schoeninger 1991). Different isotopes of a single element will have different kinetic and thermodynamic properties when they undergo chemical reactions though, because of differences in reaction rates and heat capacity influenced by their different atomic masses (Urey 1947). So, while isotopes of a like element will react the same chemically, they will react at different rates, due to their different atomic masses and sizes. Different metabolic and chemical processes therefore change the ratios between the isotopes in a characteristic manner (van der Merwe 1982). It is also noted that as atomic weight increases, the differences in thermodynamic properties between isotopes generally decrease (Urey 1947). In other words, light isotopes such as those of

hydrogen, carbon, and oxygen will have a much greater variation in their thermodynamic and kinetic characteristics than heavier isotopes such as strontium and lead.

Stable isotopes are not radioactive (Hoefs 2004), thus they do not spontaneously change into another atom or another isotope of the same element (Herz & Garrison 1998). Revisiting the carbon example, when considering the three isotopic forms of carbon (^{12}C, ^{13}C, ^{14}C), the former two are stable isotopes, while the latter is radioactive (van der Merwe 1982), and commonly utilized for archaeological dating purposes.

Stable isotopes may also be characterized as radiogenic or nonradiogenic. A particular isotope is classified as radiogenic if it is the product of the decay of a "long-lived" radioactive isotope (Schwarcz & Schoeninger 1991). Strontium (^{87}Sr) and lead (^{206}Pb, ^{207}Pb, ^{208}Pb) are the primary radiogenic isotopes used in nutritional ecology studies. ^{87}Sr forms from the radioactive decay of rubidium (^{87}Rb) while ^{206}Pb and ^{207}Pb arise from the decay of uranium (^{238}U in the case of ^{206}Pb and ^{235}U for ^{207}Pb) and ^{208}Pb results from the decay of thorium (^{232}Th) (Herz & Garrison 1998). These radiogenic isotopes vary considerably in abundance with respect to their associated non radiogenic isotopes (^{86}Sr and ^{204}Pb) (Schwarcz & Schoeninger 1991) and serve as useful analytical tools.

Eighty-one elements have stable isotopes of varying numbers (Herz & Garrison 1998). All of the biochemically important elements, with the exception of fluorine, have more than one stable isotope (Schwarcz & Schoeninger 1991). Four of these; carbon, oxygen, strontium, and lead; were examined in this study will be discussed in detail being on page 6.

Measurements of stable isotopic ratios are performed by a mass spectrometer, an instrument that determines the relative abundances of different isotopic masses in a variety of elements (Thirlwall 1997). For carbon, the mass spectrometer determines the raw ratio of $^{13}C/^{12}C$, which it then compares to the ratio of a marine carbonate standard, known as Pee Dee belemnite (PDB, now referred to as V-PDB, based on the Vienna Convention; Hoefs 2004). The difference between the sample ratio and the V-PDB standard ratio is what is known as the relative ^{13}C content and is the value reported and used for inferential purposes (van der Merwe 1982). The equation is as follows:

$$\delta_{element} = (ratio_{sample}/ratio_{std} - 1) \times 1000‰ = \text{value in } ‰$$

$$\delta^{13}C = \left(\frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{V\text{-}PDB}} - 1 \right) \times 1000‰ \qquad (1\text{-}1)$$

This measure is denoted by the symbol δ (delta) and measured in parts per mil (‰) (van der Merwe 1982). If the hypothetical $^{13}C/^{12}C$ ratio of a sample was calculated as 12 per mil less than the V-PDB standard, the $\delta^{13}C$ value would be -12‰ and considered depleted compared to the sample. It is important to note that the V-PDB standard does not equal zero (it equals 2.0671×10^{-6}; Hoefs 2004) and results should not be interpreted as deviations from the zero point.

Oxygen values for $^{18}O/^{16}O$ are calculated similarly. When $\delta^{18}O$ is calculated in concert with $\delta^{13}C$, the V-PDB standard is used along with a conversion factor (Dr. Jason Curtis, personal communication). When isotopic calculations are performed singly or in combination with hydrogen, the internationally accepted standard of standard mean ocean water (SMOW or V-SMOW) is used (Hoefs 2004). The heavy isotopes of strontium and lead are not generally normalized to a conventional standard, but instead, results are

expressed directly as ratios (Herz & Garrison 1998) and the standards are used for mass spectrometer calibration adjustments.

Stable isotope standards have been drawn from a variety of sources over the years. Some of the most commonly utilized in zoological and anthropological studies are listed in Table 1-1.

Table 1-1. Stable isotope standard materials and calibrants.

Element	Ratio	Standard (Std)	Std Notation	Std Value
Hydrogen[1]	D/H ($^{2}H/^{1}H$)	Standard Mean Ocean Water	SMOW or V-SMOW	155.76×10^{-6}
Carbon[1]	$^{13}C/^{12}C$	*Belemnitella Americana* from the Cretaceous Peedee formation, South Carolina	PDB or V-PDB	2067.1×10^{-6}
Nitrogen[1]	$^{15}N/^{14}N$	Air nitrogen	N_2 (atm)	3676.5×10^{-6}
Oxygen[1]	$^{18}O/^{16}O$	Standard Mean Ocean Water also	SMOW or V-SMOW	2067.1×10^{-6}
		Belemnitella Americana from the Cretaceous Peedee formation, South Carolina	PDB or V-PDB	2067.1×10^{-6}
Strontium[2]	$^{87}Sr/^{86}Sr$	Strontium carbonate/bulk earth	NBS-987 or NIST 987	0.7045
Lead[3]	$^{208}Pb/^{204}Pb$ $^{207}Pb/^{204}Pb$ $^{206}Pb/^{204}Pb$ $^{207}Pb/^{206}Pb$ $^{208}Pb/^{206}Pb$	Lead metal wire	NBS-981 or NIST 981	36.696 15.491 16.937 0.9146 2.1665

[1] From Hoefs (2004)
[2] From Beard and Johnson (2000)
[3] George Kamenov (2006)

Study Isotopes

Carbon

In 1968, Margaret Bender first reported that the major photosynthetic pathways of plants manifest themselves in distinct carbon isotope ratios. This discovery served as the catalyst for the multitude of carbon isotope studies documented in the literature today. When interpreting carbon isotope signatures, one must harken back to the days of basic biology class and discussions of the differences in the two major photosynthetic systems.

C_3 photosynthesis occurs in the majority of cultivated and wild plants in temperate regions (Schwarcz & Schoeninger 1991), such as wheat, rice, and barley, and produces an initial three-carbon metabolite (van der Merwe 1982, Schwarcz & Schoeninger 1991, MacFadden et al. 1999b). C_4 photosynthesis, found in more drought-resistant plants, produces an initial four-carbon compound in cultigens such as sugar cane, maize and millet (van der Merwe 1982, Schwarcz & Schoeninger 1991, MacFadden et al. 1999b). These different metabolic processes produce different isotopic ratios, which are then incorporated into plant tissues. C_4 plants exhibit more rapid carbon dioxide intake leading to values between -9‰ and -16‰. C_3 plants on the other hand, have slow rates of carbon dioxide uptake leading to values from -20‰ to -35‰ (van der Merwe 1982).

What makes carbon isotope analyses so powerful is that these to ranges do not overlap. Intermediate values are found in plants utilizing a third photosynthetic pathway, CAM or crassulacean acid metabolism (van der Merwe 1982, MacFadden et al. 1999b). These plants are primarily succulents such as cactus and pineapple, and as such, they neither factor significantly into most human diets nor the present research.

Plants demonstrate preferential uptake of ^{12}C to ^{13}C, thus they are depleted in ^{13}C compared to ^{12}C (Bender 1968). These two carbon species are differentially incorporated

into body tissues (i.e., they are fractionated in a characteristic manner) during digestive processes (Durrance 1986). As a result of the differences in photosynthetic pathways in plants, it is also possible to determine approximate proportions of C_3 versus C_4 plants in an individual's diet based on the $\delta^{13}C$ value (Schwarcz & Schoeninger 1991).

Carbon isotopes also convey information regarding the use of marine foods in an organism's diet. Marine animals present isotopic signatures intermediary to C_3 and C_4 food chains (Schoeninger & DeNiro 1984, Larsen et al. 1992). Marine mammals and fish display $\delta^{13}C$ values that are enriched by roughly 6‰ over animals that feed on C_3 foodstuffs, and depleted by about 7‰ compared to animals that feed on C_4-based foods (Schoeninger & DeNiro 1984). The best indicator of a reliance on marine food sources is the information provided through a joint $\delta^{13}C$ and $\delta^{15}N$ analyses (Schoeninger & DeNiro 1984, Ambrose & Norr 1993).

Oxygen

Oxygen is the most abundant elemental component of the earth's crust (Herz & Garrison 1998) and its isotopic ratios provide an indication of the point of origin of remains. Isotopes of oxygen take the form of ^{16}O, ^{17}O, and ^{18}O (Mattey 1997). Oxygen is primarily incorporated into body tissues via atmospheric oxygen, water, and oxygen bound in food (Sponheimer & Lee-Thorp 1999b). Because the $\delta^{18}O$ value of atmospheric oxygen is relatively constant, it is believed that oxygen isotopic signatures are primarily representative of imbibed water, and to a lesser extent, the macronutrients found in foodstuffs (Sponheimer & Lee-Thorp 1999b). The oxygen isotopes in water are preserved in bone, teeth, and other tissues and are reflective of a particular environment and climate, decreasing with increasing latitude, increasing altitude, and as you move inland (Dupras & Schwarcz 2001, Kendall & Coplen 2001, Rubenstein & Hobson 2004).

Analytically available oxygen is present in both the phosphate and carbonate ions of hydroxyapatite in the mineral phase of skeletal tissues. Most studies have examined phosphate oxygen because the P-O chemical bond is much stronger than the C-O bone, suggesting that phosphate oxygen is less susceptible to diagenesis than carbonate oxygen (Iacumin et al. 1996, Sponheimer & Lee-Thorp 1999b). Lengthy and harsh chemical procedures are required to extract the phosphate oxygen from apatite however, while the carbonate portion is easily obtained from the CO_2 produced during mass spectrometry for carbon isotopes (Sponheimer & Lee-Thorp 1999b). Bone carbonate has shown a strong positive correlation to local meteoric water with an r^2 value = 0.98 (Iacumin et al. 1996). Additionally, both carbonate and phosphate are better preserved by highly-mineralized tooth enamel versus more porous dentin and bone phosphate (Iacumin et al. 1996).

Strontium

Strontium has been used to characterize prehistoric mobility patterns since the mid 1980s (Budd et al. 2004, Millard et al. 2004). There are four stable isotopes of strontium: ^{88}Sr, ^{87}Sr, ^{86}Sr, and ^{84}Sr. Only ^{87}S is the product of radioactive decay (radiogenic), being a product of the beta decay of rubidium 87. This radioactive decay pair, $^{87}Rb \rightarrow ^{87}Sr$, has consequently produced distinctively different ^{87}Sr abundances in different parts of the earth over its history (Beard and Johnson 2000) that have proven quite valuable in tracing the origin of matter to a particular locale.

Strontium signatures depend purely on local geology since they reflect the underlying bedrock of a particular area. Strontium isotopic ratios vary with the age and type of bedrock underlying the soil. So the quantity of strontium in a particular rock will depend not only on the amount of rubidium parent material found in the rock, but the age of the rock as well as the original amount of ^{87}Sr present in the rock when it was formed.

Strontium varies in plant tissue with the age and type of geological substrate or bulk composition. Older soils are more enriched compared to younger soils as are calcium-rich soils compared to calcium-poor soils. Additionally, atmospheric deposition or dry fall from natural sources can also affect strontium values (Beard and Johnson 2000). Anthropogenic factors that can influence isotope ratios include nuclear fallout, airborne pollution from fossil fuels, and land-use practices that expose bedrock (Rubenstein and Hobson 2004).

Strontium is incorporated into human tissue following the calcium pathway because this non-nutrient, non-toxic element has chemical properties similar to calcium (Åberg et al. 1998). During nutrient uptake strontium often replaces calcium in bones and therefore can be used to trace the flow of minerals from the soil through the food web (Rubenstein and Hobson 2004). "Strontium concentrations in plants and animals are controlled by trophic position, but the isotopic composition is invariant; that is, Sr does not fractionate. Thus, bones and teeth in an individual will have different Sr abundances but identical $^{87}Sr/^{86}Sr$ ratios" (Herz & Garrison 1998), with human enamel demonstrating lower strontium content than bone (Price et al. 1994, Grupe et al. 1997, Beard & Johnson 2000). If food sources are local then, all participants in the food chain, regardless of what tissue is sampled, should reflect the same isotopic signature.

Additionally, strontium abundance has commonly been examined to discriminate between the meat and vegetable components in an organism's diet. Toots and Voorhies (1965) published the seminal study in this area, discovering significant differences (p-value <0.001) not only between the mean strontium concentrations for fossil Pliocene carnivores and herbivores, but among the herbivorous grazers and browsers themselves.

The basis for this is that for each trophic level above the soil, there is a metabolic discrimination against strontium in mammalian epithelium, as opposed to calcium (Radosevich 1993). As one increases in trophic levels among the consumers, the contribution from food sources to skeletal strontium is decreased at each step (Toots & Voorhies 1965). Plants will retain 50-100% of the strontium found in the soil, with each progressive trophic level exhibiting a reduction of 33% strontium over the lower level (Radosevich 1993). Keep in mind that this refers to strontium abundance (or concentration) and not the $\delta^{87}Sr$ value. So theoretically, someone such as a vegan should have a higher strontium concentration than an ardent follower of the Adkins' diet, by approximately 33%. Radosevich (1993) cautions against blindly accepting these measurements however, without first considering factors such as parent material and soil chemistry variation influencing plant uptake and physiological differentiation, as well as behavioral changes in feeding strategies, trophic placement, and cultural practices.

Lead

"Lead is one of the most heavily utilized metals in human history" (Sangster et al. 2000). Lead has four naturally occurring stable isotopes: ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb. As previously discussed, the latter three isotopes are radiogenic. Because ^{204}Pb is not radiogenic, it serves as stable reference isotope (Sangster et al. 2000). Similar to strontium, the isotopic composition of lead in a particular locale (or ore deposit) is dependent upon four factors: 1) the length of time before lead was separated by geological processes in the source reservoir; 2) the decay rate of the parent isotopes; 3) the initial ratio of the abundance of the parent material to the abundance of lead in the source reservoir; and 4) the initial isotopic constitution of the reservoir lead (Sangster et al. 2000). The variations in parent isotope decay rates result in systematic differentials in

the ratios of ^{206}Pb, ^{207}Pb, and ^{208}Pb to each other, as well as to ^{204}Pb (Sangster et al. 2000). Most archaeological studies are based on the ratios of the radiogenic isotopes to ^{204}Pb, whereas environmental studies tend to also form ratios from only the radiogenic isotopes themselves (Dr. George Kamenov, personal communication). Additionally, lead is favored by many researchers because like strontium, it does not exhibit fractionation in nature (Stille & Shields 1997).

Lead is assimilated into skeletal elements in a similar manner to strontium, in that it accumulates from the blood through calcium pathways and substitutes for calcium in the carbonate hydroxyapatite fraction of hard tissues (Vogel et al. 1990). Juveniles exhibit a higher propensity to absorb ingested lead than adults (Reinhard & Ghazi 1992), likely due to the rapid modeling of bone occurring during the growth phase and because small children tend to frequently put objects in their mouths. Lead particles are thought to enter the body through ingestion, either through food stuffs/fluids or lead objects, or inhalation (Gulson 1996). Environmental contamination by lead is found through mining operations, waste dumps, emissions from lead smelting, coal combustion, and leaded gasoline (Åberg et al. 1998). Furthermore, acid rain can transmit contamination from emissions/combustion over great distances.

Fractionation

Prior to drawing conclusions regarding the delta value of a material, additional issues such as fractionation effects must be factored in. Fractionation is the disparate partitioning of isotopes between two substances or tissues (Hoefs 2004). Without it, biological processes would be homogenous and some of the most powerful inferences in isotopic analyses would not be possible.

Differential fractionation manifests itself in a variety of forms. One example is the different rates carbon is fractionated as one progresses through the food chain. Carbon found in the atmosphere is present with a near constant $^{13}C/^{12}C$ ratio of about 1:99 (Chisholm 1989). As plants incorporate carbon into their tissues during photosynthesis, isotopic fractionation occurs altering the $^{13}C/^{12}C$ ratio. Since C_3 and C_4 photosynthetic pathways differ chemically, they produce different degrees of fractionation. This is beneficial, and in fact, essential in the case of carbon isotope studies, because the $\delta^{13}C$ values can be utilized to classify between C_3 and C_4 plants and diets based on a complete separation of approximately 14‰ between groups allowing for discrimination between them (DeNiro & Epstein 1978a, 1978b, Chisholm 1989, Ambrose & Norr 1993).

The selective metabolism and recombination of plant chemicals within organisms feeding upon them, results in fractionation of elemental isotopes, leading to differences in $\delta^{13}C$ values between diet and bone collagen of primary consumers of +3‰ to +5.3‰. An additional fractionation factor of about +1‰ must be accounted for as you increase in trophic level (i.e., from primary to secondary consumer) (Schoeninger 1985, Chisolm 1989, Schoeninger 1989, Ambrose 1993). The $\delta^{13}C$ values of mammal hydroxyapatite trend even further from the whole diet, with rats on experimentally controlled diets showing an enrichment of +9.6‰ (DeNiro & Epstein 1978b) and other mammals displaying enrichments of +12‰ to +13‰ (Lee-Thorp et al. 1989). Additionally, preferential uptake among different tissues within the same organism has been noted and can further complicate matters, with animal muscle generally showing $\delta^{13}C$ values 3‰ to 4‰ less positive (-3‰ to -4‰) compared to bone collagen (Schoeninger 1989).

Oxygen undergoes fractionation due to environmental factors such as evaporation, condensation, and freezing and is also strongly influenced by temperature and humidity (Stille & Shields 1997, Iacumin 1996, Hertz & Garrison 1998, Kendall & Coplen 2001). This leads to differential isotope incorporation in plant tissues and is reflected in the differing values of herbivores thought to be a result of foraging habits. For instance, oxygen isotope ratios were found to vary by as much as 8‰ to 9‰ in herbivores based on whether they were browsers or grazers (Iacumin et al. 1996).

A difference of approximately 9‰ has also been measured between the carbonate and phosphate fractions of bone and teeth from a variety of mammals (Iacumin et al. 1996) as well as marine invertebrate shells (Longinelli & Nuti 1973). This consistent enrichment of carbonate $\delta^{18}O$ values, regardless of the animal, seems fairly constant as long as temperature remains within the range of 0^{O} C to 37^{O} C. Outside of this temperature range, the fractionation is not as predictable (Iacumin et al. 1996).

One reason strontium and lead analyses appear so attractive is the general consensus that these elements do not undergo fractionation in nature. Strontium and lead do not appear to exhibit this trend due to their significantly large atomic masses (Stille & Shields 1997) versus the lighter isotopes such as carbon and oxygen. Such being the case, comparisons can be drawn then utilizing organisms from different trophic levels as well as between different tissues, without having to employ conversion factors.

Frequently Sampled Human Tissues

A variety of human tissues have proved useful in isotopic studies within the anthropological disciplines in recent years. Tissues primarily available to forensic anthropologists include bone, teeth, hair, desiccated skin, and finger/toenails; each

presenting its own benefits and drawbacks potential isotopic use and preserving records of residency and diet at different points of the individual's life.

Bone

Bone is arguably the most utilized tissue in archaeological isotope studies (Schwarcz & Schoeninger 1991). It is a composed of three primary constituents: 1) water; 2) an inorganic mineral fraction (hydroxyapatite); and 3) an organic matrix (Schwarcz & Schoeninger 1991). Bone isotope studies utilize both hydroxyapatite (apatite) and collagen, which is found in the organic phase. Dry bone is composed of approximately 70% inorganics and 30% organics (Katzenberg 2000). The overwhelming majority of the inorganic phase is comprised of the protein collagen (85% to 90%) (Katzenberg 2000).

Bone has a turnover rate of between 10–30 years (Ambrose 1993) owing to the fact that different bone components remodel at different rates. On average, trabecular bone remodels much more rapidly than its denser cortical counterpart (Teitelbaum 2000). Regardless of the speed of turnover, it is clear that bone delta values slowly change throughout an individual's life as stable isotopes are constantly incorporated into this continually remodeled tissue.

Apatite is a calcium phosphate product of which the carbonate portion arises from dissolved carbon dioxide (CO_2) in the blood plasma. Fractionation does occur between these two reservoirs with the bone carbonate portion $\delta^{13}C$ value enriched by approximately 12‰ over plasma CO_2 (DeNiro and Epstein 1978b). Bone carbonate therefore reflects the total metabolic carbon pool found in an individual's diet, incorporating carbon equally from all dietary energy sources and representing the isotopic signature of the whole diet (DeNiro and Epstein 1978b, Ambrose & Norr 1993).

Collagen is the most abundant protein in the body (Champe & Harvey 1987), constituting roughly one-quarter of all proteins occurring in mammals (Stryer 1975). The collagen found in bone, dentin, skin, and tendon is molecularly similar and falls under the category of Type I collagen (Schwarcz & Schoeninger 1991). Controlled experiments using rats demonstrated that collagen underestimates the non-protein component of the diet, but is an excellent representative of the protein portion because of its heavy nitrogen constituent (Ambrose & Norr 1993, Tieszen & Fagre 1993). One difficulty with bone collagen is that is does degrade over time, much more so than apatite.

Teeth

Teeth are especially useful in isotopic studies because of their robustness and ability to survive in environs where bone would normally degrade. Unlike bone, tooth enamel tends to be highly inert in terms of mineral exchange with the environment (Price et al. 2002, Lee-Thorp & Sponheimer 2003), consequently they represent small, closed systems. Because enamel is non-cellular and heavily mineralized with 96% or greater of the weight of the enamel comprised of the inorganic constituent (Hillson 1996), it withstands the effects of diagenesis very well and long preserves an accurate biogenic isotopic signal (Lee-Thorp & Sponheimer 2003). Dentin and cement, on the other hand, are much heavier in organics (roughly 20% and 25%, respectively) (Hillson 1996) and much more susceptible to contamination.

Additionally, the inorganic nature of enamel, and specifically the apatite, reflects the whole diet of the individual while the collagen in dentin, because of its high nitrogen content, primarily mirrors the protein content of the diet (van der Merwe 1982, Harrison and Katzenberg 2003). This is one of the drawbacks of using enamel. You cannot analyze nitrogen isotopes.

Moreover, since teeth are genetically conservative, there is little variation in the development and specifically, the period of mineralization of the tooth, although females are slightly precocious in terms of dental formation, completing most stages of dental growth before males (Fanning & Brown 1971, Hillson 1996). This observation was confirmed by the 1976 study by Anderson et al. of the mineralization in permanent dentition, although the authors state the degree of variability between the sexes has been reported to be similar. Their calculations of the mean age of attainment of mineralization in the adult teeth are presented below in Table 1-2.

Table 1-2. Mean age of completion of permanent crown mineralization.

	1st Incisor	2nd Incisor	Canine	1st Premolar	2nd Premolar	1st Molar	2nd Molar	3rd Molar
Males								
Maxillary	3.7±0.28	4.0±0.48	4.9±0.53	5.8±1.0	6.3±0.65	3.8±0.30	6.7±0.72	13.3±1.58
Mandibular	3.6+0.21	4.0±0.46	4.8±0.59	5.6±1.21	6.3±0.70	3.7±0.14	6.7±0.71	13.3±1.51
Females								
Maxillary	3.6±0.14	3.8±0.40	4.1±0.49	5.1±0.56	5.9±0.65	no data	6.3±0.66	12.7±1.49
Mandibular	3.6±0.20	3.7±0.28	4.1±0.49	5.0±0.54	5.9±0.74	no data	6.3±0.66	12.8±1.63

Source: Anderson et al. (1976)

With in- and outflow of materials ceasing once amelogenesis is complete, examining the permanent enamel provides a snapshot of the nutritional ecology of that individual during the period of crown mineralization for that specific tooth. Dentin primarily is laid down during and after amelogenesis, thus the bulk of it is formed during childhood. Secondary dentin lines the pulp chamber and has a slow, continued formation during adulthood, with turnover rates similar to bone (Hillson 1996).

Sampling can be done in bulk, which will average the isotope value for the entire tissue component, or serially. In serial analyses, very specific regions of the enamel or dentin, corresponding to even finer time periods, are sampled and compared. This takes much greater skill in drilling and one must be sure what point in the individual’s life the

area represents, but this method can also allow even finer resolution of dietary studies over a period of years.

Hair

Several studies have turned to hair as an alternative sampling tissue (van der Merwe et al. 1993, Yoshingaga et al. 1996, O'Connell & Hedges 1999, White et al. 1999, Bonnichsen et al. 2001, Ayliffe et al. *2004*, Cryan et al. 2004, West et al. 2004, *Roy* et al. 2005). Hair holds a great untapped potential in forensic isotope work. Often, hair masses are found in association with skeletal remains. It is extremely durable, proving insoluble to a variety of fluids, and can remain intact for thousands of years (Bonnichsen et al. 2001). The shaft is sheathed in a cuticle, a hard protective covering that is resistant to chemical and microbial insult (Lubec et al. 1987, Macko et al. 1999b).

Because of its hardiness and the fact that the average human sheds 50-100 hairs a day (Macko et al. 1999b), sampling is easy and essentially non-invasive. Non-keratinous material, such as the root (bulb) is not normally sampled (Ayliffe et al. *2004)* because of its signature is not reflective of the shaft. Hair is also readily renewable, growing roughly 1 cm/month in humans (Yoshinaga et al. 1996), with isotope shifts demonstrating about an 8-day delay (in the case of beard hair) from change of diet to hair exposure from the follicle (Sharp et al. 2003). The isotopic composition of hair offers information concerning an individual's diet and recent geolocational background. Thus a section of hair provides a snapshot of an individual's nutritional ecology at a particular point in time and a chronological record of the same along its length (White et al. 1999, Roy et al. 2005).

Hair is easier to isotopically analyze than bone and only very small samples (much less than bone) are required (Cryan et al. 2004, Roy et al. 2005). Hair is made of

approximately 95% keratin, the proteinacious component (Taylor et al. 1995). Because of its high protein content, hair requires minimal chemical processing and no chemical purification. Conversely, bone and dentin collagen must be chemically extracted and purified before the protein fraction can be analyzed (Ambrose 1993).

Roy et al. (2005) produced consistent carbon and nitrogen isotope values with human hair specimen weights as low as 100 µg, corresponding to a length of 2 cm of hair. The authors expect that strand lengths as small as 5 mm could be analyzed without significant loss of precision when determining $\delta^{13}C$ alone, as nitrogen was the limiting factor in their study.

The $\delta^{13}C$ values of hair keratin correlate well with that of total dietary protein, with keratin being enriched by +1‰ to +4.8‰ relative to protein in the diet and depleted by -2‰ to -3‰ compared to bone collagen (DeNiro & Epstein 1978b, Ambrose & Norr 1993, Tieszen & Fagre 1993, Yoshinaga 1996) in lab animals and contemporary humans. Carbon isotope signatures from hair keratin and bone collagen are related but cannot be directly equated (O'Connell & Hedges 1999).

Fingernails and Toenails

Like hair, finger- and toenails also offer a non-invasive way to examine isotopic values in both the living and dead. The keratin composition of human nail material makes them an excellent source of collagen values, as well as a variety of elemental isotopes. They also provide a recent geolocational reference for a specific individual with a whole nail representing approximately 6 months of growth in adults (note: authors did not state the length of the nail) (Fraser et al. 2006) and 2 to 3 months growth from cuticle to fingertip in infants (Fuller et al. 2006a). While some state that hair and fingernail values are "similar" to each other (O'Connel et al. 2001, Fuller et al. 2006a) , it

has been noted that nails are depleted in ^{13}C and ^{18}O compared to hair by mean values of and -0.55‰ and -1.6‰, respectively (Fraser et al. 2006).

Fogel et al. (1989, 1997) published the details of a landmark study examining the weaning of modern infants as reflected in the differences in $\delta^{15}N$ between mothers and infants. Fingernails prove an excellent medium for studying diets in modern infants because they are metabolically inert, resistant to degradation, and have such a fast synthesis rate (Fuller et al. 2006a). What the authors found was that the isotopic values of the babies' fingernails were enriched in ^{15}N by approximately +3‰ from that of their mothers, indicating that the infants were feeding at a higher trophic level than their mothers. This was confirmed by Fuller et al. (2006a), who found infant $\delta^{13}C$ values enriched by +1‰ over their mothers' and ^{15}N enrichment of +1.7‰ to +2.8‰ compared to maternal values (for more detail, see Chapter 2). Such conclusions have been extrapolated to bone and tooth isotopic analyses of weaning practices of archeological populations (Schurr 1997, Herring et al. 1998, Schurr 1998, Wright & Schwarcz 1998, Wright & Schwarcz 1999, Dupras et al. 2001, Mays et al. 2002, Clayton et al. 2006, Fuller et al. 2006b).

Skin

Often desiccated skin is adherent to bone on remains submitted for forensic analysis. This skin serves as an additional potential reservoir for isotopic values and can be relatively easily removed from associated bone. Carbon turnover rates for skin and hair are much faster than for bone, giving these tissues the ability to confer information regarding diet and provenance much closer to death than hard tissues. Skin has an estimated carbon turnover rate of roughly 15 days (Tieszen et al. 1983). The integrity of skin after the decomposition process takes hold however is suspect, as skin appears

highly susceptible to contamination (White et al. 1999). For those studies in which viable skin samples were obtained, relative to hair sample $\delta^{13}C$ values, skin appears to be consistently depleted from -0.2‰ to -2.7‰ (White & Schwarcz 1994, White et al. 1999)

Complications

Radosevich (1993) aptly states that a reason for uncritical acceptance of methods or assumptions is often the simple desire for a new technique to work. Stable isotope analyses have seemingly been hailed as near-omniscient and people may turn a blind eye to the limitations of such methods. On the other hand, modeling biological systems is an extremely complex undertaking. There are times when a reductionist approach can overwhelm the model in minutiae; where accounting for all the potentials of error eclipses the actual data. All factors with the potential for confounding the data need to be explored and understood, but often a relative weight can be assigned to them so the model is not overloaded. There are much potential for error in stable isotope analyses; but, as long as they are recognized *a priori* and dealt with, isotope ratios can provide valuable insight into past and present systems.

Diagenesis

After the initial glow wore off following the popularization of stable isotope techniques, researchers began to find chinks in the analytical armor. Often confusing or contrary results were obtained leaving researchers to scratch their heads as to what it all meant and if isotope studies were really worth all the hype. In 1981, A. Sillen was one of the first to propose that perhaps post-depositional contamination, or diagenesis, was responsible for at least a portion of this noise, but the effects of diagenesis were largely ignored or dismissed in most studies (Price et al. 1992).

Diagenesis is a subset of the study of the postmortem processes which can affect bone appearance and integrity, commonly known as taphonomy (literally meaning the "laws of burial;" Sandford 1992). These processes take both physical and chemical forms. When diagenesis in an anthropological context is discussed, it is in reference to the postmortem alterations in the chemical constituents and physical properties of bone following deposition in soil. Diagenesis takes the form of both contamination and leaching and arises from several different mechanisms (Sandford 1992).

The dense mineralization of enamel affords teeth a great measure of protection against effects, but it is important to keep in mind that no skeletal element is impervious to postmortem modification. The porous structure of bone however, makes it susceptible to infiltration by foreign elements, especially when it has been physically degraded. The intrinsic skeletal chemistry and microstructure of osseous tissue therefore leads to a dynamic relationship between it and the environment in which it is interred (Sandford 1992).

Mary Sandford (1992) lists several different means by which the environment interacts with the structure of bone, leading to alteration:

- Elements may be precipitated as discrete "void-filling" mineral phases in the small cracks and pores of bone.

- Soluble ions present in soils may be exchanged for those that normally occupy lattice positions in bone hydroxyapatite.

- Bone apatite can "seed" formation of recrystallization through a variety of means.

- Microorganisms break down bone collagen releasing elements through its dissolution and the action of acid metabolites on hydroxyapatite.

Additional extrinsic factors such as the chemical environment of the burial sight and the properties of the enveloping sediment influence the incidence and rate of processes as well. Soil pH is one of the most important variables that affect change in bone. Gordon and Buikstra (1981) first quantified the relationship, determining that it is strongly negatively correlated, thus as soil pH decreases, degradation of bone increases. The authors also noted that skeletal age was significant as well, with juvenile bone being more susceptible to decay.

Temperature, microorganismal activity, groundwater, and precipitation also play a role, as does the local geochemical environment to include soil texture, mineralogy, and organic content. Sandford (1992) also mentions further intrinsic factors bearing on processes such as bone density, size, microstructure, and biochemistry.

Recent investigations have shed light on bone alteration leading to several generalizations: 1) elements differ in their susceptibility to diagenesis; 2) certain categories of bones are more susceptible to diagenesis--less bone density, greater porosity, or large quantities of amorphous material may predispose certain classes of bones, such as immature bone, to taphonomic processes; 3) denser cortical bone withstands diagenesis much better than the lattice-like trabecullar bone; 4) Direction and intensity of change is not necessarily temporally or spatially uniform (Sandford 1992); 5) the color and condition of skeletal material can be used as a general indicator of the degree of diagenesis (Carlson 1996). The more the color approximates the color of fresh bone, the less likely it is to have undergone change.

The majority of changes seen in bone arise due to precipitation of authigenic carbonate or other minerals, exchange reactions in original carbonate or phosphate, and

uptake or loss of various trace elements. Recrystallization can also occur, producing various phosphate-containing compounds with trace levels of elements often replacing calcium at higher concentrations than found in modern bone (Schoeninger et al. 2003).

The same processes that bring about diagenetic change are ones that will eventually return bones the lithosphere. The overwhelming majority of all deposited skeletal material disappears relatively quickly, especially if exposed to taphonomic factors such as acidic soil, alternate wet/dry conditions, strong solar radiation, and/or injurious invasion by microorganisms (Lee-Thorp 2002). If we as anthropologists are fortunate to encounter remains in the first place, we should not be discouraged from utilizing isotopic resources in attempting to uncover clues about the lifestyle of the individual(s). We must keep in mind that these processes are not uniform over space and time, and thus even old remains can produce valuable results.

Questions still remain however, as to what measures can be taken to minimize the impact of diagenesis on isotopic interpretation. So what is a researcher to do? The first step is to attempt to determine if processes have occurred and to what extent. In reality, these processes are always occurring, but whether they exact a measurable effect upon bone is another question. To begin with, a scientist should ask themselves several questions. The first is what is/are the element(s) of interest? Studies show that isotopes of such elements as strontium and lead are little changed in bone due to diagenetic means (Beard and Johnson 2000, Carlson 2002), thus scientists should have greater latitude in using bones that have been interred for any period of time. Do the bones belong to an adult or child? Because smaller bones have greater surface area to volume ratios, they are more susceptible to change since there is more surface area for processes to act upon.

The absolute volume of cortical bone is reduced in juveniles as well, as they are still growing, so bones are less shielded from environmental assailants. What bones are available for sampling? Remains higher in cortical bone preserve better, so if presented with a few cranial vault fragments, a researcher may be wise to opt out of isotope analysis versus if a femoral shaft is available. Also, intact bone is always preferable to fragmentary bone.

One should also assess the environment the bones are interred in. Sandford (1992) believes chemical analysis of soil is a mandatory requirement for gaining insight as to the condition of bone. Soil samples should be recovered from feature fill in direct association with bone (Gordon and Buikstra 1981). Samples can be prepared and pH determined *in situ* utilizing a portable pH meter. These values can then be applied to something similar to a regional variant of Gordon & Buikstra's (1981) regression formulae for pH and state of preservation. (It is interesting that while the authors provide several regression formulae, for example, in adult assemblages, preservation = -1.3(pH) +12.5, there is no scale provided in which to interpret the preservation value.)

Further testing can compare total elemental concentrations of bone and associated soil. Following the assumptions of the concentration gradient theory, significant contamination of bone by soil is considerably less likely if soil concentrations of a specific element are disproportionately different than those same elements in bone. If a more homogenous elemental state has been reached between bone and soil, it is a good indication that significant change has transpired (Sandford 1992, Carlson 1996).

Other factors such as temperature and exposure to water should also be accounted for. It is well accepted that higher temperature leads to degradation of collagen and that

warm, moist habitats encourage microbial proliferation. Exposure to water can also lead to increased rates of both contamination and leaching of minerals into the surrounding soil. The best environs for the preservation of DNA are those that are cool, dark, and dry (Smith 2005). That is because these same conditions optimize the resilience of the whole bone complex, so isotopic fractions will be best preserved as well. Heavy bone erosion, trauma, burning, associated human alterations such as boiling and internment/funeral practices, and carnivore and rodent activity compromise the structural integrity of the bone itself leaving it more vulnerable to processes.

Instrumental analyses can be completed as well to include electron microprobes and x-ray diffraction (Sandford 1992), and backscatter scanning electron microscopy (Collins et al. 2002). These methods attempt to look at the structure of bone and analyze it for changes in crystalline architecture, chemical constituency, and microbial activity. Analyses of collagen content of bone may also provide insight, since some have observed low yield in collagen is often associated with aberrant stable isotope readings (Katzenberg 1992).

Osteological comparisons can also be completed in conjunction with soil analyses examining constancy in values (Sandford 1992). Intrabone comparisons look for statistically significant correlations between elements and known contaminants or “indicator elements.” Interbone comparisons look for agreement with the assumption that different types of bone, such as ribs and femora, should reflect varying degrees of diagenesis. Interspecies comparisons can indicate activity when measured elemental values vary from those predicted on the basis of dietary patterns. Additionally, if

interpopulational data were available as we are attempting to collect, congruency to published values could be ascertained (Sandford 1992).

Further precautions are essential during sample preparation in the laboratory. Standard protocols attempt to minimize effects of diagenesis through mechanical abrasion, to physically remove contaminants from outer bone surfaces, and acid washing. None of the aforementioned methods are fail safe, but their use enhances overall understanding of the processes active in a certain area and attempts to circumvent diagenetic effects by careful sampling selection and preparation.

Many subscribe to the notion that the longer a set of remains has been interred, the greater the alteration to the material. It is unwise to use temporal criterion in isolation in making a decision about employing isotopic analyses though. As in any scientific situation, you must take measure of as many variables as possible in order to make the most informed decision. Diagenesis is a complex mechanism and time is but one factor that comes into play. Cases in the literature abound detailing the successful extraction of viable isotopic material from fossils that would have proved opportunities lost if the authors had decided against isotopic analyses simply because they were working with very old material. Studies have examined diets in ancient, human mummies (White & Schwarcz 1994, White et al. 1999) and Neolithic Icemen (Macko et al 1999a, 1999b; Müller et al. 2003), and the diet and paleoecology of *Australopithecus africanus* (van der Merwe et al. 2003) and 5 million-year-old horses (MacFadden et al. 1999b), to cite but a few. Differential preservation is a rule, rather than an exception and thus each interment must be individually assessed for the appropriateness of isotopic analyses.

Anthropogenic Contamination

All organisms alter their environment. Human beings are unique though, in that we are the only species on the planet that is actually altering the basic conditions of life on Earth (Vitousek et al. 1997). We have altered landscapes, climate, and biogeochemical cycles. Many of the wastes generated by our industrial metabolism play no useful role in nature, cannot be recycled (i.e., nuclear waste) or overwhelm the current processing capabilities of the biosphere (McMichael 2001). The ecological footprint of the human species is enormous. Everything we do leaves traces of our kind behind.

This anthropogenic effect extends to isotopic signal variation. Industrial pollution is implicated in the changing of isotopic values when contemporary populations are compared to paleological assemblages and can outright alter or mask the isotopic signatures a researcher is attempting to interpret. This can complicate analyses and lead to false conclusions if not identified. To account for this, several correction factors have been established to ease temporal analyses. Because nearly all of the anthropological work done with stable isotopes has been in bioarchaeological contexts, these corrections are essential in drawing conclusions.

The carbon isotope ecology of terrestrial systems is controlled by atmospheric carbon dioxide (van der Merwe et al. 2000). This has changed dramatically in the years since the Industrial Revolution, with fossil fuel emissions altering the $^{13}C/^{12}C$ ratio of the atmosphere by -1.5‰ in the last 150 years. (van Klinken et al. 2000). To correct for this change in $\delta^{13}C$ values, "Industrial Effect" (van der Merwe et al. 2000) or "fossil fuel effect" (van Klinken et al. 2000) calibrations must be factored into results, normally by adding 1.5‰ to convert modern samples to pre-industrial values (van Klinken et al. 2000).

We also have significantly altered the lead content of certain environs. Budd et al. (2000) state, “It is widely believed that the contamination of the atmosphere by anthropogenic lead has led to far greater human exposure today than that which prevailed in the distant past, but this has proved difficult to quantify.” A marked increase in the mobilization of lead in Europe and North America occurred after industrialization. Drilling of Greenland ice-cores has revealed a ten-fold rise in lead concentration, with rates skyrocketing from roughly 10 parts per billion (ppb) to 100 ppb in the last 100 years (McMichael 2001). This is due primarily to environmental contamination due to the use of leaded gasoline (which is still utilized in many nations), lead-based pigments and compounds, lead-acid batteries, and through mining operations, soldering, and coal combustion (Sangster et al. 2000).

Global Economy

Today’s global economy has the potential to homogenize biogeochemical signatures in contemporary people. Because of world-wide trade, especially when it comes to food importation, what people eat may not necessarily reflect where they came from. Strontium values are especially vulnerable to being washed out by the effects of the global food market. Archaeological research does not usually concern itself with such matters because food tended to be locally grown and consumed. After the Industrial Revolution and the establishment of global trade networks, food in the U.S. was very rarely grown in the localities were people lived. So, on a trip to the refrigerator one may find bananas from Guatemala, grapes from Chile, and free range, grass-fed beef from Argentina.

Increasing consumption of bottled water from non-local sources further complicates matters, affecting not only strontium values, but oxygen and hydrogen as

well. This situation may be further complicated by the importation of fertilizer produced in foreign countries (Price et al. 2002). Such soil additives will affect not only plant intake but run-off will affect, and may significantly change, the isotopic values of groundwater (Böhlke & Horan 2000).

CHAPTER 2
APPLICATIONS OF STABLE ISOTOPE ANALYSES

Examples of the varied usages of isotopes in the literature abound. Stable isotope analyses are an extremely effective means of recreating paleoecology (e.g., Amundson et al. 1997, Cerling et al. 1997), tracking animal movements (e.g., Burton and Koch, 1999 Rubenstein & Hobson 2004), assessing migratory patterns of humans (e.g., Beard and Johnson 2000, Dupras and Schwarcz 2001), and determining diet (e.g., DeNiro & Epstein 1978, van der Merwe 1982, MacFadden et al. 1999b). Within anthropology, stable isotope analyses have been primarily relegated to realm of archaeology, but by applying the technologies currently used in geology, paleontological and modern zoology, and archaeology to forensic science, an effective means for presumptive identification emerges.

Tracing Studies

One exciting application of stable isotopes that transcends disciplinary bounds is that of tracing studies. In a tracing study, an element is introduced into a system with a known delta value and tracked through the system or at the termination of certain processes to see how that element normally moves through the system. This approach is frequently used in clinical nutrition studies to understand the uptake of various nutrients (see Abrams 1999 for a review). Stable isotopes offer many benefits over more traditional radioactive approaches in that they present little of a safety concern for pregnant women or children and are less difficult and less expensive to remove than radioactive wastes (Abrams 1999).

For instance, isotopic tracer studies were used to measure the efficiency of zinc utilization at different doses. Patients were given labeled zinc solutions, and then urine samples were collected to determine absorption rates. Based on this approach the study concluded aqueous zinc doses greater than 20mg resulted in quite small and diminishing increases in absorptivity (Tran et al. 2004). Magnesium tracer studies demonstrated that absorption of isotopically labeled magnesium could be accurately monitored through urine sampling versus more invasive blood and fecal sampling methods (Sabatier 2003). Additionally, tracer studies in Nigerian children with rickets determined that those with the disease did not express impaired abilities to absorb calcium when compared to healthy counterparts, although fractional calcium absorption did increase after resolution of the active disease (Graff et al. 2004.) Stable isotopes were even utilized to measure calcium metabolism of two cosmonauts and one astronaut aboard the Mir space station prior to, during, and after a 3-month spaceflight (Smith et al. 1999). Further non-human trials utilized three diets of different isotopic compositions to determine the turnover time of carbon isotopes in horse tail hair West et al. 2004) and tail hair and breath CO_2 (Ayliffe et al. 2004). These baseline studies could then be applied to other wildlife studies in an attempt to understand the dietary history of mammals

Ecological studies have also utilized isotope tracers to examine nutrient flow in various systems. One recent study added isotopically-labeled nitrogen to a creek for 6 weeks and monitored ^{15}N in dissolved, aquatic, and terrestrial riparian food web components. High levels of incorporation of the tracer into the tissues of resident organisms led researchers to believe that streams within undisturbed primary forests may be highly efficient at uptake and retention of nitrogen (Ashkenas 2004). Another project

examined root turnover in relation to forest net primary production by fumigating a stand with labeled ^{13}C in the form of $^{13}CO_2$ over a 5-year period, then sampling fine roots. Their results suggest that root production and turnover in forests have likely been overestimated and that sequestration of anthropogenic atmospheric carbon in forest soils may be lower than currently believed (Matamala et al. 2003).

Fractionation Studies

Fractionation studies have proven quite illustrative in a variety of genres as well. Examination of carbon and nitrogen stable isotopes has yielded greater understanding of the decompositional processes found within soil organic matter (Kramer et al. 2003). Fractionation studies have also proven useful in attempting to measure the contribution of gluconeogenesis to glucose production in humans. Here, body water was enriched with ^2H_2O and the ratio of ^2H bound to carbon-5 versus carbon-2 of blood glucose was measured (Katanik et al. 2003). Oxygen isotope fractionation has also been employed in niche separation studies of African rain forest primates occupying overlapping microhabitats. Oxygen isotope ratios from bone carbonate were positively correlated with relative dependence of leaves in the diet, a fact obscured by carbon isotope analyses (Carter 2003). A final study led to the discovery of what is commonly known as the "canopy effect" (van der Merwe &Medina 1991). Van der Merwe and Medina discovered the re-use of plant-fractionated, respired CO_2 in dense vegetation can cause systematic bias between plant and animal species living on the forest floor versus those living in the forest canopy and open environments (also in van Klinken et al. 2000). Due to the "canopy effect," the $\delta^{13}C$ value of atmospheric CO_2 is lowest near the forest floor. "Leaves fixing this ^{13}C-depleted CO_2 have lower $\delta^{13}C$ values than those higher up in the canopy. Combined with the effects of low light intensity, high humidity and high CO_2

concentrations on water use efficiency, this creates a vertical cline in leaf $\delta^{13}C$ values" (Ambrose 1993).

Zoology and Ecology

Within zoology and ecology, the examples of stable isotope use seem limitless. One of the first such studies examined carbon ratios of two sympatric fossil hyrax species, determining one a was browser, based on the C_3-like signature these animals displayed, while the other was chiefly a grazer, feeding on tropical grasses, which utilized a C_4 photosynthetic pathway (DeNiro & Epstein 1978a). Similar studies have shed new light on the diet and ecology of 5-million-year-old horses (MacFadden et al. 1999b) and Cenozoic sirenians from Florida (MacFadden et al. 2004). Stable isotopes have proven especially insightful for scientists attempting to determine feeding strategies of marine organisms, and in fact, "Most work on mammal and reptile movements using stable isotopes has been done in the marine environment" (Rubenstein & Hobson 2004). Carbon isotopes have been used to determine food sources for Red Sea barnacles (Achituv et al. 1997) and examine photosymbiosis in fossil mollusks (Jones et al. 1988). Delta ^{13}C and $\delta^{15}N$ were useful in assessing not only the foraging strategies of Pacific pinnipeds (Burton & Koch 1999), but tracking their migratory movements as well and have been used in dietary studies of North Atlantic bottlenose dolphins (Walker et al. 1999). Moreover, adult female loggerhead turtles were sampled from around Japan to determine the relationship between body size and feeding habitats (Hatase et al. 2002).

Claws (Bearhop et al. 2003) and feathers (Rubenstein et al. 2002, Bowen et al. 2005) have also been utilized to determine diets and habitat use of migratory birds whose summering and wintering grounds are separated by thousands of kilometers; so too has hair been examined in bats for evidence of seasonal molt and long-distance migration

(Cryan et al. 2004). Wing membranes from monarch butterflies have been sampled for hydrogen and carbon isotopes to identify natal regions within the United States and revealed that 13 discrete wintering colonies in Mexico were fairly well mixed as to the origins of the individuals (Wassenaar & Hobson 1998). Biogeochemical fingerprints of African elephant bone have been assessed to determine change in diet and habitat use (Koch et al. 1995). Isotopic analyses have even been extended to determining the allocation of reproductive resources in butterflies (O'Brien et al. 2004) and assessing prey quality in predatory spiders (Oelbermann & Scheu 2002).

Stable isotope ratios also allow us a glimpse into the past. Based on $\delta^{13}C$ enamel values of worldwide fossil mammals and modern endemic and zoo-housed African mammals, Cerling et al. (1997) has postulated that between 8 and 6 million years ago, there was a global shift to increased C_4 plant biomass and a corresponding decrease in atmospheric carbon dioxide. This has implications today as increasing levels of atmospheric carbon dioxide could bring about a major biotic alteration towards a world dominated by C_3 plants, which would have widespread ecological consequences. Carbon values have also been analyzed from ancient pollen in attempts to reconstruct paleovegetational and paleoclimatic conditions with the hope of someday tracing the origin of the C_4 photosynthetic pathway (Amundson et al. 1997).

One further study has taken an innovative approach to tying stable isotopes to hominin evolution. Wynn (2004) examined paleosols of Turkana Basin, Kenya, and ascertained that modern hominins evolved during a period of waxing and waning diversity of savanna-adapted fauna in an environment that trended towards increasing aridity. Those hominins best suited to generalization of resources were the most capable

of surviving through evolutionary “pruning events” as savanna ecosystems changed through time.

Mention of these zoological and ecological studies does not even scratch the surface as to the diversity of isotopic studies that have been and are continuing to be conducted within these disciplines. Isotope use in these fields is gaining momentum and results generally enjoy widespread acceptance, ensuring their continued use far into the future.

Archaeology

Within anthropology, stable isotope analyses have primarily been relegated to the realm of archaeology. Here, they have been used extensively to answer a litany of questions in a variety of contexts concerning the human experience.

Diet Assessment

Introduction of maize

In archaeological contexts, stable isotopes have been used extensively to infer diet, mobility patterns, and origins of material culture. When considering diet, a great amount of effort has been expended attempting to determine when exactly maize became prominent dietary component in various human populations (Vogel & van der Merwe 1977, van der Merwe & Vogel 1978, DeNiro & Epstein 1978b, Farnsworth et al. 1985, Norr 1995). In fact, the majority of early archaeological stable isotope studies were aimed at resolving the temporal and geographic origins of maize introduction, especially into North America (Schwarcz & Schoeninger 1991). Striking changes in $\delta^{13}C$ values of collagen resulted from the introduction of maize into human dietary patterns. These values markedly decreased from roughly -21.4‰ to -12.0‰ during the period of A.D. 1000–1200 indicating that proportion of carbon from C4 plants went from 0 to more than

70% in some individuals (van der Merwe & Vogel 1978, van der Merwe 1982). This agrarian shift also had other implications, with the development of permanent settlements and an abandonment of the hunter/gatherer life strategy and all associated changes inherent in the transition to a sedentary lifestyle. Not all agree on the timing of the introduction of maize to North America though, with individuals such as Farnsworth et al. (1985) concluding that maize was incorporated into human diets much earlier than indicated in the fossil record. Today, the consensus seems to be that there is a temporal variation in the conversion to maize agriculture within North America (Schwarcz & Schoeninger 1991). Age effects in a prehistoric maize horticultural population (Ontario Iroquois) have also been examined, with significantly higher $\delta^{13}C$ values found in infants and young children suggesting a weaning diet high in maize (Katzenberg et al. 1993).

Isotopic dietary studies have been applied to fossils as old as *Australopithecus africanus*, where individuals demonstrated an unusually varied diet, a large portion of which was C_4-based (Sponheimer & Lee-Thorp 1999a, van der Merwe et al. 2003). From this information, the authors speculate that by about 3 million years ago, hominins had become savanna foragers for a significant part of their diet. Based on carbon and nitrogen stable isotope values, the diet of a Neolithic Alpine “Ice Man” was determined to likely be primarily vegetarian, at least in the period closest to his death, based upon hair values (Macko et al. 1999a).

Stable isotope dietary studies have also been performed on individuals from other Mesolithic/Neolithic sites (Krigbaum 2003, Richards et al. 2003, Milner et al. 2004), the Bronze Age in Northern Jordan (Al-Shorman 2004), prehistoric Chile (Macko et al. 1999b), preclassic and historic Mayan Belize (White & Schwarcz 1989, Tykot et al.

1996), ancient Egypt (Macko et al. 1999b, While et al. 1999) and Sudan (White & Schwarcz 1994), prehistoric South Africa (Sealy et al. 1992, Lee-Thorp et al. 1993), and prehistoric Micronesia (Ambrose et al. 1997). In addition, indigenous Easter Islanders (Fogel et al. 1997), additional Native North American groups throughout time (Price et al. 1985, Larsen et al. 1992, Fogel et al. 1997, Hedman et al. 2002, Roy et al. 2005, Yerkes 2005), and colonists from the Chesapeake area (Ubelaker & Owsley 2003) have also been examined.

Weaning practices

One significant aspect of diet that has received much recent attention is that of infant feeding. Breastfeeding practices, to include weaning, have wide implications for population dynamics in earlier human groups (Mays et al. 2002). Breastfeeding is a major determinant of fecundity and interval between births in societies lacking reliable artificial contraceptive measures, (Vitzthum 1994, as cited in Mays et al. 2002) and thus, can be a major factor in determining life histories of certain population groups. Ultimately, the success of infant feeding will have far-reaching impacts in terms of population health and growth, for it is the essential first step for realizing adulthood.

Bone chemistry has been critical in this area for archaeological interpretation of remains. In 1989, Fogel et al. published a groundbreaking study comparing the fingernails of mothers and newborns from birth through weaning, to determine the utility of using isotopes for such analyses. Fetuses and newborns have a $\delta^{15}N$ roughly equivalent to that of their mothers (Herring et al. 1998, Mays 2000). This makes sense because a fetus receives nutrition through materials exchanged across the maternal and fetal circulatory flows in the placenta. Once born and breastfeeding begins, neonates change their trophic stratigraphy. They effectively become carnivores relative to their

mother (Ambrose 1993). Nursing infants are feeding at one trophic level above their lactating mothers and hence, should show an enriched $\delta^{15}N$ level of +2‰ to +4‰ over their mothers (Fogel et al. 1989, Fogel et al. 1997). This is exactly what Fogel et al. found. During the period of breastfeeding, they measured infant ^{15}N ratios approximately +3‰ higher than their mothers (Fogel et al. 1989, Fogel et al. 1997). These results were further confirmed by Fuller et al. (2006a), who found infant δ15N values enriched by +1.7‰ to +2.8‰ compared to maternal values. As a child is weaned from its mother's breast, its $\delta^{15}N$ level will begin to fall back towards a standard adult average, because the shift from milk proteins to proteins obtained from solid foods registers as a decrease in ^{15}N bone collagen values (Wright and Schwarcz 1998, Fuller et al. 2006b).

Since Fogel et al. (1989), numerous researchers have applied their findings to various archeological assemblages ranging from mid-Holocene South Africa (Clayton et al. 2006), the Roman period of Egypt (27 BC to AD 395) (Dupras et al. 2001), Mediaeval England (Mays et al. 2002), pre-contact North America (Schurr 1997) to 19th century Ontario (Herring et al. 1998). Wright and Schwarcz (1998, 1999) have taken a slightly different slant by including oxygen isotopes in their analyses of prehistoric Guatemalans. Their studies are based on the fact that, "Human breast milk is formed from the body water pool and, thus, is heavier in $\delta^{18}O$ than the water imbibed by a lactating mother" (Wright & Schwarcz 1998). Infants who only breastfeed are enriched in their oxygen ratios compared to their mothers, because of the mothers' metabolic processing of the water incorporated into breast milk (Wright & Schwarcz 1998, Wright & Schwarcz 1999). Additionally, many studies have incorporated carbon delta values with the standard nitrogen values to determine the approximate ages of supplementary food

consumption by children in their respective populations, providing further validation of the conclusions drawn from nitrogen data. (Wright & Schwarcz 1998, Clayton et al. 2005, Fuller et al. 2006b)

Following in the footsteps of Fogel et al. (1989), many more contemporary studies have been carried out in the hopes of applying the results to archaeological work. O'Connell et al. (2001) compared pairings of hair keratin and bone collagen taken from patients undergoing orthopedic surgery in the United Kingdom as well as pairings of nail and hair keratin from living subjects to examine the utility of applying similar $\delta^{13}C$ and $\delta^{15}N$ results to archaeological work. Lead isotopes in modern people have also been examined to determine comparative lead loads and digenetic effects in prehistoric teeth (Budd et al 1998, Budd et al. 2000), with Budd et al. (2000) concluding that Neolithic human enamel lead values were only an order of magnitude lower than modern juveniles.

Region of Origin

Paleodiet analyses have also been applied to detect human mobility since the mid-1980s (Sealy & van der Merwe 1985, 1986). Such studies are predicated upon the notion that individuals practicing seasonal migration from coastal to inland areas should have similar $\delta^{13}C$ values, while those permanently inhabiting such diverse areas should demonstrate distinct carbon ratios (Sealy & van der Merwe 1985, 1986). In addition to carbon, there are a wide variety of isotopes that can be drawn on to infer information concerning human migration. Schwarcz et al. (1991) were the first to demonstrate the use of bone phosphate $\delta^{18}O$ to in attempting to identify the geographical origin of 28 soldiers from the War of 1812, interred in the Snake Hill cemetery, New York. Their findings of uniformity among $\delta^{18}O$ values indicated the group all spent a major portion of their lives living in the same geographical area. These values differed however from

oxygen isotope analyses performed on interments in southwestern Ontario and Antietam, Maryland. Dupras and Schwarcz (2001) used oxygen isotopes to distinguish immigrants from native peoples from a third-century cemetery in the Dakhleh Oasis, Egypt, and both oxygen and strontium isotopes have been used to determine the geographic origin of remains found from Viking occupation-era graves in Great Britain (Budd et al. 2004).

Strontium isotope ratios have been used extensively in transhumance studies from Neolithic Europe (Grupe 1997, Budd et al. 2000, Bentley et al. 2002, Bentley et al. 2003, Müller et al. 2003, Bentley et al. 2004) as well as Bronze Age and Romano-British sites (Budd et al. 2000a, Montgomery et al. 2005, Fuller et al. 2006b), and prehistoric and historic South Africa (Sealy et al. 1995). Two studies used strontium isotopes to discriminate between immigrants and life-long residents of 14th century Grasshopper Pueblo, Arizona (Price et al. 1994, Beard & Johnson 2000). Beard and Johnson (2000) determined local strontium values by analyzing local field mice. Individuals outside of this range were deemed immigrants to the area, with those having the greatest δ^{87}Sr differences being the most recent additions to that area. Åberg et al. (1998) demonstrated that strontium and lead isotopes could definitively distinguish between west coastal and rural inhabitants of Medieval Norway. The authors further concluded that Medieval residents subsisted on local products while contemporary people relied on imported or industrially processed food to a greater degree.

Carlson (1996) discovered that lead isotope values corresponding to different sources of anthropogenic and natural lead can indicate cultural affinity among Native Americans and fur traders buried in a 19th century fur trade cemetery. Montgomery et al. (2005) found lead to be a bit ambiguous, with results suggesting that lead isotopes

provide dissimilar types of information depending on what era is being examined. In some instances it seemed to serve as a geographical marker, while in others it served better as a cultural indicator.

Material Culture

Not all archaeological applications of isotopic signatures are anatomically-based. The origins of various forms of material culture have also been traced using these techniques and well as additional dietary analyses. The earliest attempt to determine provenance through isotope use was attempted in 1965 by Robert Brill and colleagues on lead and glass artifacts (Brill & Wampler 1967, Herz & Garrison 1998). Not only were lead objects associated with specific mining regions in antiquity, but samples separated by nearly a millennium in time were found to have virtually identical lead isotopic signatures and are believed to have come from the same mine (Brill & Wampler 1967).

The source quarries of ancient marbles have been interpreted through $\delta^{13}C$ and $\delta^{18}O$ values (Craig & Craig 1972) and today, an extensive database exists for the isotope values of principle classical quarries so marble items can now often be associated with the areas in which they originated (Herz & Garrison 1998). Oxygen values have traced emerald trade routes from the Gallo-Roman period through the 18th century (Giuliani et al. 2000) and the mining locations of lead artifacts, such as musket balls and coils, found among Omaha Native Americans have been identified (Reinhard & Ghazi 1992).

Building materials such as the timbers for the prehistoric great houses of Chaco Canyon, New Mexico, have been traced to their individual mountain growing areas (English et al. 2001). Major constituents of prehistoric and historic diet have also been accomplished through the analysis of cooking residues found on potsherds or within intact kitchenware (Hastorf & DeNiro 1985, DeNiro 1987, Hart et al. 2003). When

carbon and nitrogen analyses are combined for proven plant encrustations, they can distinguish among three plant groupings: 1) legumes; 2) non-leguminous C3 plants; and C4 or CAM vegetation (Hastorf & DeNiro 1985).

Forensic Investigations

Stable isotope analyses have been applied to a wide variety of contexts within the forensic sciences. Within Europe two major organizations have emerged to advance the development and application of isotopic work in this field. The Forensic Isotope Ratio Mass Spectrometry (FIRMS) network and the Natural Isotopes and Trace Elements in Criminalistics and Environmental Forensics (NITECRIME) European Union Thematic Network both aim to raise awareness of the benefits of isotopes to forensic investigations, encourage collaboration, and develop and validate new methodologies (Benson et al. 2006).

Stable isotopes have shown great promise as an analytical asset in the war on drugs, specifically in determining the origin of illicit narcotics. The $\delta^{2}H$ (also denoted as δD), $\delta^{13}C$, $\delta^{15}N$ of components extracted from 3,4-methylenedioxymethylamphetamine or "ecstasy" have shown that individual tablets can be traced back to a common batch (Carter et al. 2002). Carbon and nitrogen isotopes have been further used to link heroin and cocaine samples to the four major geographic regions in which they are grown (Mexico, Southwest Asia, Southeast Asia, and South America). Morphine, which is derived from heroin, demonstrated the most pronounced regional difference (Ehleringer et al. 1999). Further studies were able to determine the country of origin in 90% of 200 coca-leaf samples, the source material for cocaine, as deriving from Bolivia, Columbia, or Peru (Ehleringer et al. 2000).

Isotopic techniques have been used by food and spirit regulatory agencies as well to ensure quality control. There is an international concern with not only simple validation of food label claims, but with food adulteration as well. One application is within the beer industry (Brooks et al. 2002). The primary ingredients in beer are water, malted barley, hops, and yeast. All other "non-essential" ingredients are called adjuncts. In many nations, the use of unlabelled adjuncts is forbidden by law. Carbon delta values have proven very effective at detecting adjuncts and testing brewers' claims as to the purity of their ingredients (Brooks et al. 2002). Additionally, $\delta^{13}C$ values have proven invaluable in determining whether forms of glycerol are animal or vegetal in origin (Fronza et al. 1998) and $\delta^{13}C$ and $\delta^{15}N$ values of eggs have been used to establish whether chickens were given animal or plant protein as feed (Rossmann 2001). Furthermore, oxygen values have been utilized to verify the regional origin of dairy products, especially certain cheeses, which must be produced from milk of a particular region (Rossmann 2001).

Food adulteration is of concern to authorities because it is essentially the misrepresentation of an altered foodstuff as an authentic product. Here, a premium food product is extended or completely replaced with cheaper materials, yet fraudulently sold as a higher-end item (Parker et al. 1998). Stable isotope analyses have established themselves as a particularly usefully analytical methodology in fighting this trend. The most advanced applications of stable isotope analyses are within wine quality control, where the European Union has established an official wine stable isotope parameter database (Rossmann 2001). Carbon isotopes can detect the addition of exogenous glycerol deceptively added to wine to disguise poor quality (Calderone et al. 2004). They

have also been used to differentiate between whiskies and assist in authenticating specific whisky products (Parker et al. 1998) and determine the botanical origin of Brazilian brandies (Pissinatto et al. 1999). Isotopic fractionation of hydrogen and oxygen resulting from juice concentration processes have also been documented and utilized to quantify added sugars in orange and grape juice (Yunianta et al. 1995); while $\delta^{13}C$ values have been used for over 20 years to control for the authenticity of honey (Rossmann 2001).

Stable isotope analysis has also been employed by criminal investigators in cases involving the use of firearms (Stupian et al. 2001). Bullet individualization via lead isotope analysis was first reported in 1975 (Stupian 1975). Lead isotopic information can indicate whether a fatal bullet shared a common origin with a box of ammunition collected from a suspect or provide a detective with a tool independent of standard ballistic methods to potentially link bullets from multiple crime scenes (Stupian et al. 2001). In instances where there is a shoot-out with several types of firearms and/or ammunition, it may even be possible to conclude which bullet and/or weapon caused a particular gunshot entry (Zeichner et al. 2006).

Another forensic isotope breakthrough occurred in 1975, when Nissenbaum reported $\delta^{13}C$ could distinguish between trinitrotoluene (TNT) samples originating from different countries. Other areas of forensic isotope applications include connecting the sources of automobile (Deconinck et al. 2006) and architectural paints (Reidy et al. 2005), packaging tapes (Carter et al. 2004), and glass fragments (Trejos et al. 2003) to crime scenes.

Similar measures have been drawn upon to detect environmental toxins in soils, waters, and plants. Isotopes can assist in identifying a geographical relationship between

a source and a spilled product, whether the contamination might be from an oil spill, illegal dumping, pipeline breaks, or leaking storage tanks (Philip et al. 2003). For instance, in the case of a crime scene, such applications may be able to link engine oil on the victim of a hit and run with a particular vehicle (Philip et al. 2003). Source identification of environmental perchlorate contamination has been performed with chlorine and oxygen isotopes (Böhlke et al. 2005). Perchlorate, in even small amounts, can adversely affect thyroid function by interfering with iodine uptake (Böhlke et al. 2005), but hopefully, by identifying the source of such chemicals, this form of pollution can be stemmed.

These techniques have further been extended to biowarfare defense efforts. Horita and Vass (2003) determined that cultured bacteria (*Bacillus globigii* and *Erwinia aglomerans*) faithfully inherit the isotopic signature of hydrogen, carbon, and nitrogen from the media waters and substrates they were grown on, proving "stable-isotope fingerprint" can be created for chemical and biological agents. Because of these properties, Kreuzer-Martin et al. (2003) were able to undertake sophisticated tracing studies involving oxygen and hydrogen isotopes. Culture media was prepared with water spiked with known isotopic quantities of hydrogen and oxygen. The $\delta^{18}O$ and δD found within strains of *Bacilus subtilis* spores grown on this media were then traced back to specific water sources establishing that the origin of microbes can be pinpointed to particular areas based on the water content of the media on which they are grown.

Stable isotopes are also prominent in wildlife forensic issues. Several studies have used a trivariate approach, combining carbon, nitrogen, and strontium isotope ratios to create geolocational fingerprints for elephant ivory and bone (van der Merwe et al. 1990,

Vogel et al. 1990). It is hoped this will aid in conservation efforts by assisting in efforts to stem the illegal trade of ivory. Similar goals are also being applied to the bounty of information concerning animal migrations (Bowen et al. 2005).

While great advances have been made in the applications of stable isotope analyses to the forensic sciences, human stable isotope studies in the medico-legal realm are relatively recent phenomena. To date, very few studies examining stable isotope ratios as they pertain to region of origin in contemporary human populations have been presented or published. When examining the literature, it appears that the bulk of isotopic research in modern humans is in the form of isotopic tracers for nutritional studies (see also Abrams and Wong 2003, Mellon and Sandström 1996). Many, as previously discussed, are also used as proxies for archaeological comparison (Fogel et al. 1989, O'Connell et al. 2001, Fuller et al. 2006a).

Several studies have been conducted to investigate lead exposure and identify the sources of lead absorbed in contemporary, living children by examining their deciduous teeth (Alexander and Heaven 1993, Gulson & Wilson 1994, Gulson 1996) and other tissues and excretions (i.e., blood and urine, Angle et al. 1995). Alexander and Heaven (1993) measured $^{206}Pb/^{207}Pb$ ratios and lead abundance in teeth finding significant difference among the lead isotope ratios. When compared against various environmental sources of lead, the authors were able to identify differences in sources in northwest England. While these studies were not utilized for geolocational purposes, they nonetheless could be applied as such, (although anthropological studies tend to utilize isotopes compared to ^{204}Pb), and provide a good example of the multiple uses for isotope data.

One weaning study went one step further than those previously discussed and has exciting forensic potential. Fuller et al. (2006) analyzed bovine milk-based and soy-based formulas to determine if unique isotopic signatures exist that could identify infants being fed different forms of supplementation. The authors purchased seven different formulas sold within California and found that while the $\delta^{13}C$ values overlapped between formulas derived from cow's milk and soy, the soy products demonstrated significantly lower $\delta^{15}N$ values. This again, is a reflection of trophic level effects in nitrogen values.

Fraser et al. (2006) have begun compiling a database of modern human hair and nail values examining the stable isotopes of hydrogen, carbon, nitrogen and oxygen. The authors sampled hair and fingernails from 20 individuals living in Belfast, Northern Ireland for a minimum of 6 months as well as an additional 70 individuals from 9 countries representing 4 European nations, Syria, the United States, Australia, India, and Sudan. They did not report having yet applied the database results to a forensic situation, but preliminary data is at least at the ready should the need arise. Similarly, at the 3rd European Academy of Forensic Science Meeting in Istanbul, Turkey, Cerling et al. (2003) presented results of a multi-element study of modern human hair. The authors discovered regional differences in the δD, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of long-time residents of particular locations and appear to still be collecting samples.

Beard and Johnson (2000) were the first to demonstrate the utility of strontium isotopes in a human forensic setting. In their paper, they determined the region of origin of an illegally harvested deer using the $^{87}Sr/^{86}Sr$ ratio of antler, then also applied this information in an attempt to differentiate between the teeth of three commingled Americans associated with the Vietnam conflict. They were able to match the natal area

of one individual, but the two others presented overlapping values. If the study had also utilized alternative isotope comparisons, perhaps the authors might have been able to discern between the remaining two individuals.

Also, preliminary data for a study using strontium isotope values in an attempt to determine the geolocational fingerprints for Mexican-borne individuals residing in the U.S. was presented at the 2005 annual meeting of the American Academy of Forensic Sciences (Juarez 2005). Several bay-area dental clinics provided the author with 25 permanent 1st molars of individuals originating from four different Mexican states. Samples were accompanied by information as to the subjects' regions of origin within Mexico, their ages, and sex. Initial results indicate four specific ranges of strontium isotope ratios, one for each of the four states involved in the study. Within-state variation proved too great however, to discriminate location further.

Additionally, a presentation at the 2001 annual meeting of the American Association of Physical Anthropologists addressed the use of strontium isotopes and its applications in forensic science (Schutkowski et al. 2001). The abstract makes reference to the presentation of a multi-regional sample demonstrating differences in regional and local strontium isotope ratios. Bone and tooth signatures were examined to determine if mismatches of individual values with local isotope ratios demonstrate changes in domicile. The areas of study were likely western European, as the authors at the time of publication practiced in the United Kingdom and Germany. Unfortunately, it appears this data has yet to be published in a western source.

Gulson et al. (1997) detail a pilot study comparing the lead isotope values in teeth of native Australians to those of Australian migrants from Eastern and Southern Europe

(Table 2-1). While the actual data presented by Gulson et al. are not particularly useful in cases of American service members this paper does indicate lead isotope ratios have the potential to discriminate region of origin.

As can be seen from this short review, isotopic analyses and applications serve a wide variety of functions. The incredible inferential value of isotopic analyses in anthropology is clear. Examples of the power of isotopic studies abound in the literature and continued advances will only further solidify how essential their inclusion is within an anthropologist's analytical toolbox.

Table 2-1. Mean and standard deviations for selected groups of immigrant teeth (enamel).

	Australia (n=29)	CIS* (n=14)	Yugoslavia (n=13)	Lebanon (n=8)	Poland (n=6)
Mean $^{206}Pb/^{204}Pb$	16.56	17.98	18.23	17.62	18.07
SD	0.17	0.06	0.15	0.29	0.20
Mean $^{207}Pb/^{206}Pb$	0.9318	0.8664	0.8566	0.8825	0.8617
SD	0.0088	0.0033	0.0063	0.0136	0.0088

Source: Gulson et al. (1997)
*CIS denotes the former Soviet Union

CHAPTER 3
HUMAN FORENSIC IDENTIFICATION

Assuming that isotopic analyses do prove fruitful for forensic practitioners, this technique will be added to a bounty of available measures for use in the personal identification process. Those specializing in the forensic arts acknowledge that there is stratification when it comes to the probative value of identification data. In attempting to tease a name from a body, certain characteristics of the person will be much more unique and individualizing than others. The most powerful measure of identification is a positive identification, the essential component of which is the possession by the decedent of unique characteristics (Ubelaker 2000). Because these characteristics are not replicated in anyone else, they exclude all other individuals from consideration.

Even with the high resolution of DNA, the method of choice today for positive identifications tends to be dental comparisons (Col. Brion Smith, personal communication). Dental records are still consulted when available. The Computer-Assisted Postmortem Identification system (CAPMI) is based on the presence of dental restorations and has increased the efficiency of matching and comparing antemortem/postmortem records (Friedman et al. 1989), especially in the case of mass fatalities. Dental radiographic matches are much quicker and less costly than DNA evaluations, although the number of individuals with no dental anomalies (Friedman et al. 1989, Col. Brion Smith, personal communication) is rising due to advances in dental hygiene and medicine and mass fluorination of community water sources. In those cases

where the skin of the fingers is still intact, fingerprints may establish a positive identification as well.

When these measures prove inconclusive, genetic fingerprinting utilizing nuclear and mitochondrial DNA is another option. With the development of the polymerase chain reaction procedure, which enabled rapid amplification of genetic material, and lowered costs, DNA analysis is much more practical (Herrero 2003) than in days past. Nuclear DNA is known to be a unique identifier (unless the subjects are identical twins). Many investigators, including the Department of Defense (DoD), test 16 bands from the available microsatellite loci pool (Col. Brion Smith, personal communication). From experimental observations, the average odds that one band will be shared by any two unrelated individuals is approximately 0.25 (Sudbery 2002). So the resolution of a 16-band testing procedure is $0.25^{16} = 2.33 \times 10^{-10}$; that is, there is a 0.000000000233 chance that two unrelated individuals will share all 16 bands tested. Put another way, if you take the reciprocal of this figure you see that there is a 1 in nearly 4.3 trillion chance that someone unrelated has the same DNA profile. Since this number is considerably larger than the world's population, nuclear DNA testing is said to provide for unique identifications.

This calculation is made with the assumptions that all individuals are unrelated and that the chance that bands will be shared is the same for all people. In truth, people are related and ethnic affinities may lead to higher rates of band sharing than among the general world populace. Even so, after accounting for such complications, nuclear DNA analyses are still considered positive and unambiguous identification (Sudbery 2002).

The resolution of mtDNA, on the other hand, is not as fine. Because mtDNA is passed through maternal lineages only, recombination does not occur. Mutations aside, this accounts for the integrity of mtDNA as it is passed from mother to child. This constancy of code allows for familial tracing by comparing sequences of certain base pair lengths among those who are maternally related. This is a very powerful tool indeed, and allows for a distinctive discriminating function from nuclear DNA. The downside to it though is that is cannot distinguish among relatives and can be preserved for generations, leading to populations of people with the same or similar mtDNA profile (Col. Brion Smith, personal communication).

Many also consider various forms of radiographic comparison to equate to positive identification. This is especially true with the frontal sinus. The sinus becomes radiographically visible between 7 and 9 years of age, and barring trauma or disease, remains relatively unchanged throughout life (Ubelaker 1999). In a comparison completed by Ubelaker (1999), the author noted than in a radiographic comparison of 35 radiographs (595 comparisons), no two frontal sinuses were alike. The number of differences between individuals average to approximately 8, with a range of 3 to 15. If additional antemortem radiographs exist documenting unique skeletal anomalies (i.e., pathology or trauma), these characteristics may also serve as a basis for positive ID.

One further skeletal anomaly for consideration is that of prosthetic devices (Burns 1999). While it may not be unique that an individual has a total knee replacement, what will be unique is the serial number that is imprinted upon the prosthetic device along with the manufacturer’s emblem. Hospitals must document these serial numbers. With a little detective work, the serial or lot numbers can be traced back to the manufacturer who in

turn, can direct an investigator to the hospital to which the device was sold (Warren 2003). Some also consider the comparison of still photos and the skull at the same angle, or what is known as video superimposition, to be conclusive as well (Ubelaker 1999).

Hope for a positive ID can often prove frustrating and futile though, when there are no reference samples on file for that individual. An individual must have antemortem information available to compare against if a positive identification is to be achieved. So, for instance, while DNA may have successfully been extracted from a set of remains, an identification cannot be accomplished when there is no nuclear DNA on file or source material available and no relatives of maternal lineage for the decedent can be located.

One step below a positive ID is exclusionary evidence for identification. When remains are presented for identification, they will arrive from one of two environs, either an open environment, or one which is closed (Warren 2003). Open environments are those in which the person laying before you could be anyone in the world who was up until recently, alive. For example, a body found in the woods could be an indigent, a local, or a tourist from another country. In the case of a light aircraft crash however, the potential for identification is much higher. If a passenger manifest was filed listing two adult males and child of 12, and assuming it was correct, then there is the potential for an exclusionary identification. The child will be easy to distinguish from the adults due to developmental differences in the skeleton. If one of the adults is identified via antemortem radiographs and the other has no antemortem comparison data, then the latter would usually be identified by exclusionary methods, since ideally, in a closed system there is no one else it could be.

Burns (1999) also lists identification by means of a preponderance of evidence. This is often linked with tentative identifications, or what are also known as presumptive identifications. There is much greater uncertainty with presumptive identifications because they are based on evidence found associated with the body, such as personal effects, and/or verbal testimony of witnesses, last known whereabouts of the body, and familial recollections of undocumented conditions the individual may have suffered from (Burns 1999). See Table 3-1 for a recap of identification measures.

Table 3-1. Forms of forensic identification.

Type of I.D.	Basis for I.D.
Tentative identification	Clothing Possessions Location of body Verbal testimony
Identification by preponderance of evidence	Anomalies known by family or friends, but without the existence of written records
Identification by exclusion	"Everyone else is identified and there is no evidence that this is not the only person still missing."
Positive identification	Dental identification Radiographic identification Mummified fingerprints Prosthetic identification DNA analysis Unique skeletal anomalies

Reproduced from Burns (1999)

Military Identification Measures

"Over the past 200 years, the United States has set the standard for the identification and return of its servicemembers [sic] to their families" (AFIP 2004). Since as early as the American Revolution, efforts have been made to recover, identify, and provide individual burial for American military personnel (AFIP 2004). As the years have progressed, standards and expectations for identification have increased and the technology with which to do it has made sweeping advances.

The United States DoD employs all of the standard personnel identification measures previously mentioned. What is unique about the military as a population however, is that their physical attributes and markers are much better documented than the general populace (i.e., they have much better antemortem records). Members have fingerprints on file and flight crews have footprints documented as well. Meticulous medical and dental records are fairly centralized. With few exceptions, blood cards are on file for all current total force members in the case their DNA needs to be sequenced. Individuating marks are noted such as scars, large birthmarks and moles, and tattoos as well as information such as hair and eye color, race, stature, weight, and age.

Even so, such measures are not without their complications. Dental radiographs are commonly not available of military members unaccounted for from previous conflicts, especially World War II and the Korean War (Adams 2003a). The Office of the Armed Forces Medical Examiner notes that greater than 5% of all service members have no dental restorations, the primary means for dental identifications, and the number is rising (AFIP 2004). In a study of 7030 living U.S. soldiers, it was revealed that 9% had a full complement of unrestored teeth (Friedman et al. 1989). In a pooled data set of over 29,000 individuals from the Third national Health and Nutrition Examination Survey (NHANES III) and the Tri-Service Comprehensive Oral Health Survey (TSCOSO), Bradley Adams found that 12.77% had “perfect teeth” (2003b). Not only has the number of dental restorations declined in younger individuals, but the complexity of them has decreased as well (Friedman et al. 1989).

Historically, only 70% of service personnel actually have their fingerprints on file with the Federal Bureau of Investigation with a further 15–30% of the fingerprint cards

submitted by the services rejected as “unclassifiable” (AFIP 2004). These numbers will likely be reduced significantly though, with the wide-spread implementation of digital fingerprinting DoD-wide, which instantly scans recorded images for acceptability immediately after each individual print is taken. Additionally, radiographic analyses may not be possible on highly fragmented remains (AFIP 2004). Identifications made based on material evidence associated with remains can be very problematic as well. Traditional items such as dog tags are not necessarily accurate either. As an example, during current operations in Iraq and Afghanistan dog tags have been known to have been blown off one individual and burned into the chest of another (Dr William Rodriguez, personal communication).

On the leading edge of identification efforts for the U.S. government is the Armed Forces DNA Identification Laboratory (AFDIL). AFDIL is the focal point for the DoD in all matters concerning DNA identification efforts for military personnel and special federal government projects. In addition to performing laboratory testing, AFDIL manages the DoD DNA Registry. This function is responsible for maintaining blood cards for DNA testing as well as providing administrative oversight of the database of all sequenced data. Besides the Registry, AFDIL is also responsible for the DNA Repository, which administers the AFDIL Family Reference Specimen database for mtDNA matching when nuclear DNA is unavailable (AFIP 2004).

It is a common misconception that the military maintains DNA profiles on all its personnel. It does not. Instead, AFDIL houses nearly 4.5 million blood stain cards for active duty, reserve, guard component, retired military members and additional specialized government personnel (Col. Brion Smith, personal communication).

According to Colonel Brion Smith, Chief Deputy Medical Examiner for the Forensic DNA Division, Office of the Armed Forces Medical Examiner, there are two basic reasonings behind the logic of this. The first is that it is cost prohibitive to perform DNA analyses for every member of the armed forces. It is much less expensive to house blood stain cards and generate the same information on an “as needed” basis. The second is that storing the profiles of all who serve presents an ethical dilemma, especially when it comes to who should be permitted access to the information and for what purposes. This is further complicated by the fact that medical and dental records, to included DNA information and blood cards, stay on file for 50 years after the service member retires (Col. Brion Smith, personal communication).

An additional benefit of this system is it gives examiners the option *a posteriori* to decide which test is best suited based on the conditions of the remains. If the body is in good condition, nuclear DNA would be the preferred method. If the remains are charred and disassociated, mtDNA might be most appropriate. Furthermore, a fully utilized blood card can provide 30–40 punches, allowing less common tests such as Y short tandem repeats to be completed (Col. Brion Smith, personal communication) or providing the opportunity for future testing utilizing methods that have yet to be developed or hit the mainstream. The beauty of this practice then is that technicians are not restricted to only performing a form of analysis that matches the information present in a data base so there is greater flexibility in analyses and hopefully the best method for the materials available.

The utilization of both nuclear and mitochondrial DNA is dependent upon the situation and essential to military identification. All individuals who die in current

combat, training, or in otherwise duty-related capacities are sampled for DNA analyses upon intake to the Dover Air Force Base Port Mortuary (Col. Brion Smith, personal communication), the DoD central receiving and processing center for all military deceased. Even when other conventional methods of positive identification are available such as radiographic dental comparisons, a DNA fingerprint will be generated. This will delay returning a casualty to their families unless identity is questionable, but instead, is performed to prevent questions surfacing at a later date as to correct identification and to reassure family members that the body being returned to them is kin. (Col. Brion Smith, personal communication).

Present Study

This project was established to test the utility of stable isotope analyses for identification of region of origin for modern, unidentified, human skeletal material that has poorly-documented or unknown provenience. Initial efforts have focused on the approximately 1,800 service members who remain unaccounted for from the Vietnam conflict. It is hoped however, that this information will eventually be refined to use in the identification of all those who remain unaccounted for and for those potentially recoverable from previous conflicts (Table 3-2).

Often, the true national origin of remains recovered by the Joint Prisoner of War/Missing in Action Accounting Command (JPAC) is uncertain. In addition, it is not uncommon for de-contextualized, poorly preserved and/or highly fragmented remains to be unilaterally turned over to the Central Identification Laboratory (CIL) by a foreign agency. CIL personnel attempt to determine whether remains are U.S. service

Table 3-2. Numbers of unaccounted for U.S. prisoners of war and/or those missing in action.

Conflict	Number Unaccounted For
World War II	78,000 (35,000 considered recoverable)
Korean Conflict	8,100
Vietnam War	1,800
Cold War	120
First Gulf War	1
Source: JPAC (2006)	

personnel through a variety of means. The identification of unknown remains believed to be missing U.S. service personnel is frequently hampered by high levels of degradation and fragmentation as a result of circumstances of loss and subsequent taphonomic regimes. These effects often combine to prevent effective DNA sampling strategies. Teeth often prove excellent at distinguishing among the populations in questions. U.S. military personnel had access to regular dental care. In countries such as Vietnam, this was not the case for the majority of the population. In addition to untreated dental insults, the occlusal surfaces of the molars and other teeth are frequently worn down from the grit present in native diets exposing the underlying dentin (Mark Gleisner, personal communication). The teeth then of modern Vietnamese often present similarly to historical/prehistorical Native Americans. Every effort is also made to extract DNA from a set of remains, although such efforts are often unsuccessful because of the poor state of preservation.

Additionally, the number of U.S. casualties during the Vietnam conflict of Asian ancestry was relatively small. In 1985, the DoD reported the number of "Mongolian" fatalities in Southeast Asia occurring from the period of 1 January 1961 to 30 April 1975 or as a result of injuries sustained in operations during said period was 114 or 0.002%.

Those listed of “Malayan” ancestry who died under the same circumstances was 253 or 0.004% (Reports 1985). See Table 3-3 for a complete listing of casualties by race.

More importantly for this study, only 5 servicemen of Asian ancestry remain unaccounted for out of 1,760 total (JPAC 2006). The complete racial breakdown for service members still listed as missing in Southeast Asia can be found in Table 3-4. Besides military members, 32 American civilians are also listed as missing in Southeast Asia. The racial backgrounds of these individuals were unavailable, but it is interesting to note that two missing civilians are female. All of the military members unaccounted for are male.

Because of the very low likelihood of a U.S. service member being a female or of Asian ancestry, biological profiles can be useful in excluding individuals from consideration. This is assuming enough of the skeleton remains to create a biological profile. When the biological information is combined with documented information

Table 3-3. United States casualties in Southeast Asia by race.

Race (reported by DoD)	Total U.S. Casualties
“Caucasian”	49,951
“Black”	7,257
“Mongolian”	114
“American Indian”	226
“Malayan”	253
“Other/Unknown”	221
Total	58,022

Source: Reports (1985)

Table 3-4. United States military listed as unaccounted for in Southeast Asia by race.

Race (reported by JPAC)	Total U.S. Military Missing
“White”	1,653
“Black”	92
“Asian/Pacific Islander”	5
“American Indian/Alaska Native”	2
“Other”	8
Total	1,760

Source: JPAC (2006)

concerning troop engagement and staging areas and locations of downed aircraft, remains may be returned to the originating nation if the evidence points overwhelmingly to the fact that the remains are not of an American. Unfortunately, such an assessment is an extremely complicated venture and in a great many cases it is simply impossible to make such a distinction.

This project was initiated in the hopes that the results will assist in resolving this dilemma. A two-pronged approach for this study has been utilized based on the operating hypotheses that: 1) discernable differences exist between the isotopic ratios incorporated into American and Southeast Asian tooth enamel and that these differences can be used to determine region of origin; and 2) regional differences in natal isotopic signatures are also discernable within populations raised within the U.S.

Because of the paucity of data in contemporary studies, it is near impossible to predict the likelihood of the ability of this study to distinguish natal Vietnamese from American-born individuals. It is encouraging that Juarez (2005) found significant variation among the strontium isotope values for Mexican-born peoples from four different states, even with her limited sample. If historical, human, migratory studies (Montgomery et al. 2005, Müller et al. 2003, Montgomery et al. 2000, Dupras & Schwarcz 2001, Åberg et al. 1998) are any indicator though, there is a high probability that the chosen stable isotopes will be able to discriminate between these two populations. The reasoning behind this is that the geochemical properties of different continental systems should vary significantly and this difference will be further magnified by the fairly culturally distinct dietary practices of the two populations.

None of the studies mentioned in Chapter 2 examined stable isotope use in a forensic context in any great depth. The largest sample size was Gulson et al. (1997) with 68, but it was a combined pool of permanent and deciduous teeth. This study will utilize approximately 300–600 total samples and thus will have greater power. Furthermore, all studies make mention of overlapping isotopic values which makes discrimination virtually impossible. It is hoped this tendency will be reduced by introducing multi-element analyses to forensic work. Theoretically, a multivariate approach should allow finer resolution, especially since the deposition of the elements depends largely on very different factors: carbon isotope ratios are based on cultural food preferences; oxygen on meteoric water, altitude, and distance from major bodies of water; and strontium and lead reflect the underlying bedrock and soil.

Carbon isotope ratios reflect the photosynthetic pathways of ingested plants and echo cultural food preferences. It is expected that individuals who have subsisted on a traditional, rice-based (C_3 plant) Southeast Asian diet will differ significantly in their carbon isotope signature from individuals who have subsisted on a heavier corn-and sugar-based (C_4 plants) American diet. Wild rice in the U.S. has produced results ranging from -26.3‰ to -29.7‰ (Hart et al. 2003) and purified rice starch has been averaged to -26.6‰ (Ambrose & Norr 1993). This contrasts markedly to maize (corn) values varying between -14.0‰ (van der Merwe 1982) and -11.84‰ (Hart et al. 2003) and purified cane sugar at -11.2‰ (Ambrose et al. 1997). Americans also eat a large variety of wheat products. Wheat is a C_3 plant, but it is enriched compared to rice, with bread wheat leaves measuring -23.7‰ (van der Merwe 1982). It stands to reason then that those relying on a rice-based diet, such as the Vietnamese, would exhibit more

negative carbon isotope values than their American counterparts, whose corn and sugar constituents of the diet, will shift the carbon isotope values in a less negative direction.

Due to the fractionation effects highlighted in Chapter 1, one must keep in mind that the reported values will not trend directly with plant values. Mammal hydroxyapatite will demonstrate an enrichment of +9.6% to +13% (DeNiro & Epstein 1978b, Lee-Thorp et al. 1989) over plant material. Mixtures of the dietary plant constituent will also affect an organism's overall $\delta^{13}C$ value as well as dependency on marine food resources (Schoeninger & DeNiro 1984).

Since the majority of state borders within the continental U.S. are not based on geomorphologic formations, it is unlikely that regional identification will be as straightforward. This should be partially ameliorated through a multi-signature approach. Due to the novelty of this approach, it is difficult to say with any certainty how precise regional identification of geopolitical origin will become. Based on the limited success of Beard and Johnson (2000) however, it appears natal origin within the U.S. can be narrowed down to a regional level based on major geological formations.

Because $\delta^{13}C$ values represent dietary intake, they will not indicate regional origins, since modern diets are primarily culturally based. The stable isotope ratios for oxygen, strontium, and lead on the other hand, are aptly suited for this task. It is assumed that individuals from Alaska, Hawaii, and the American territories will be identifiable. The geographical distances between these areas and the continental United States (CONUS) are vast, with a variety of different, but interrelated, environmental factors influencing oxygen isotope distribution such as latitude, temperature, altitude, coastal affinity,

precipitation patterns, and humidity (Iacumin 1996, Hertz & Garrison 1998, Kendall & Coplen 2001).

The geologic history of the major land masses nearly represents the 4.5 billion-year history of the earth (Beard & Johnson 2000). Because of this, there are large differences in the isotope compositions of different parts of the planet. relative to the analytical error of the $^{87}Sr/^{86}Sr$ measurements (+ 0.00001 to +0.00003). Within the U.S., the ages of crust varies from under 1 million years old in Hawaii to nearly 4 billion years old in areas of Michigan and Minnesota (Beard & Johnson 2000). This age effect produces significant variations in the strontium isotope composition within different regions of the U.S. and is the basis for analytical techniques attempting to discern region of origin in different peoples. Another strength of strontium is that its isotopes are thought to be little influenced by fractionation (Toots & Voorhies 1965, Ambrose 1993, Carlson 1996, Hertz & Garrison 1998, Beard & Johnson 2000, Budd et al. 2000) thus the isotope ratio remains constant from soil to top carnivore as you move through the ecosystem. Soil samples can then be checked against values to determine the geolocational origins of a tissue sample. It is difficult to speculate whether strontium isotope analyses can identify natal geolocation to the regional level in contemporary peoples. The analyses may seem fairly straight forward on the surface, but there are underlying factors for modern man that may inhibit its deductive power. Of primary concern is homogenization of strontium values due to the global food trade.

An array of geological process are also responsible for the formation of these areas, hence the bedrock composition is quite varied. Discerning among individuals reared within the CONUS will likely prove more difficult, and overlapping values are expected.

By using the three different geologically-based isotopes in concert however, it is hoped that general patterns will emerge.

In isolation, isotope delta values have limited evidentiary value and will rarely lead to any form of identification. The same could be said of other bases of identification. Clothing alone will not lead to a presumptive identification. Someone has to recognize the clothing as belonging to the decedent before it has any realized significance. If the geo-political region of origin for a set of remains could be ascertained however, it would provide a direction in which to concentrate identification efforts. In mass disasters and in closed environments, isotopes could be combined with other methods, leading to exclusionary identifications or directing where to focus further analyses for potential positive IDs. Such techniques are relatively inexpensive and quick. Isotopic analyses can be performed for under $100 at the University of Florida and in the case of enamel, can be completed in roughly 1 week's time. If stable isotope analyses are performed at the onset of the identification process, it could save countless man-hours and dollars for the military, preventing unnecessary analytical efforts if the remains are not deemed American.

CHAPTER 4
MATERIALS AND METHODS

This study is groundbreaking in that it is the first of its kind to compile a reference sample of isotopic values associated with known natal regions to be utilized in forensic work. More importantly, the information gleaned from this study will be applied in support of the Joint POW/MIA Accounting Command's mission to achieve the fullest possible accounting of all Americans missing as a result of our nation's past conflicts.

A two-pronged approach for this project was utilized based on the operating hypotheses that: 1) discernable differences exist between the isotopic ratios incorporated into American and Southeast Asian tooth enamel and that these differences can be used to determine region of origin; and 2) regional differences in natal isotopic signatures are also discernable within populations raised within the U.S.

Teeth were utilized for this project because they are much more robust than bone and little affected by diagenetic processes. This reduces the sample preparation time by several days to a week. By only examining the enamel, isotopic values can be studies for a known period of the subject's life, because in- and outflow of materials in enamel cease at the termination of amelogenesis (Hillson 1996). It is also much easier to obtain modern teeth than modern bone for sampling. When teeth are extracted, the standard protocol is to dispose of them as biomedical waste, so there is little objection to obtaining them for study. It is much more difficult to acquire samples of contemporary bone for legal and cultural reasons. Objection is further fueled by the fact that isotopic sampling is a destructive process.

Teeth are genetically conservative displaying little variation in the period of mineralization of the tooth, although females are slightly precocious (Fanning & Brown 1971, Anderson et al. 1976, Hillson 1996), with Garn et al. reporting that females were in advance of males by an average of 3% (1958). Different ethnic groups have shown slightly different timing patterns as well, but all differences whether sex-related or ethnic, equate to not much more than a few months between groups (Hillson 1996). This fact should not impact this study however, as all crown mineralization is completed prior to individuals being eligible for military service. All teeth supplied for this study had fully completed amelogenesis.

Materials utilized in this study were supplied by three different institutions. The Joint POW/MIA Accounting Command's Central Identification Laboratory (CIL), Hickam Air Force Base, HI, permitted access to their "Mongoloid hold" collection for the creation of an East Asian reference sample. The "Mongoloid hold" collection contains remains of individuals recovered from East Asia or unilaterally turned over to the CIL, whose governments have refused repatriation, once the remains were determined not to belong to U.S. service personnel. Donated contemporary teeth and surveys completed by their donors were also provided by the 10th Dental Squadron, United States Air Force Academy (USAFA), Colorado Springs, CO and the Malcolm Randall Veterans Affairs Medical Center (North Florida/South Georgia Veterans Health System) Dental Clinic, henceforth referred to as the "VA," Gainesville, FL.

Dental Protocols

Prior to utilizing live subjects in this research, all appropriate permissions were obtained and training completed (Appendix A). The USAFA Institutional Review Board (IRB) granted IRB exempt status to this project (HQ USAFA IRB FAC2005026H). Prior

to conducting this study with the VA, the protocol was approved by the University of Florida's Health Center Institutional Review Board (IRB-01 approval #474-2005), and both the VA's Sub-Committee for Clinical Investigation and Research and Development Committee. Additionally, a research template had to be created for incorporation into each study participant's electronic medical record, via the VA's Computerized Patient Record System (CPRS).

To further assist all parties engaged in this research, information binders were distributed to both dental facilities. These packets included copies of all IRB and committee approval letters, dental staff instructions, a subject identifier log, copies of all required forms, a blank and completed, example survey, background information related to this specific research project, pre-paid FedEx shipping forms (for USAFA), and a CD with all electronic media on it (see Appendix A for a reproduction of the VA binder).

This project was essentially a piggy-back study attached to the normal patient dental care of those individuals who are selected by USAFA and the VA for tooth extraction(s) for valid medical reasons. The study, in and of itself, had no bearing on whether an individual was selected for dental extraction(s). All patients scheduled for dental extraction(s) during the study period were queried as to their willingness to participate in the study. Complete inclusion of all consenting subjects cut down on bias that would be introduced with nonrandom, arbitrary sampling by the dental staff. Dental administration personnel proctored all forms. Upon receipt from the patient, they reviewed the forms for completeness and verified birth date and sex with the subject's dental records.

Patients, to include Air Force Academy cadets, active-duty military, and military retirees and/or veterans, already identified for tooth extraction for oral health reasons, were asked to participate in a brief survey (Figure 4-1) and donate their extracted teeth for analysis. The survey and, in the case of the VA subjects, associated combined Health Insurance Portability and Accountability Act/informed consent form (Appendix A) were administered upon initial intake while the patient filled out requisite preoperative paperwork. This paperwork was in addition to the normal documentation required for dental procedures.

The HIPAA form was compulsory to protect participant health information. Researchers must obtain patient authorization before they are allowed to disclose protected health information. It was required in this instance because we were requesting information such as location of residence and birth date, which cannot be ascertained from observation alone. The informed consent form was required to secure subject participation in the study. The form detailed the background, procedures, benefits and risks of the research project, and obtained witnessed, signed consent of the individual that they knowingly and voluntarily participated in the study. Dental staff were available to answer any questions and an example of a completed questionnaire and study background information (Appendix A) was made available. Survey completion and tooth donation were the only requirements of subjects.

The data acquired from each subject included:

- Date of birth
- Sex
- Race
- Tobacco product use
- Childhood diet

- Location of residence, birth to age 18
- Date of prior dental extraction at each facility (if applicable)

Joint POW/MIA Accounting Command
JPAC
"Until they are home"

Thank you for your participation in this study. Its purpose is to provide a powerful new tool to assist in identifying our fallen servicemen and women. The information you provide will be used to determine if geographic regions of the U.S. have specific isotopic signatures that become incorporated into dental tissues. We will be looking at the mineral elements in your teeth. No DNA analysis will be performed. When compared with isotopic signatures developed for geographic areas of Southeast Asia, it is hoped the information will identify the origin of unknown remains recovered by JPAC's Central Identification Laboratory. Additionally, the information gleaned from this study may prove useful in identifying remains recovered from further conflicts such as World War II and encountered in mass disasters such as airliner crashes and the events of 11 September 2001.

Instructions: Please fully answer all **8** questions. Incomplete data may exclude your teeth from the study. If you have any questions, please ask your attending dental staff.

For Dental Staff Use Only
Sample identifier ______________
Position(s) in arcade ______________
Date of extraction ______________

1) Date of birth (day/month/year) ______________

2) Sex (circle one) **Male** **Female**

3) What race do you consider yourself? ______________

4) Have you ever regularly used tobacco products (i.e. cigarette, chew, snuff)? **Yes** **No**
If yes, what products did/do you use, what was the time period, and what was the frequency (i.e. 1 pack a day)?

	Tobacco Product	Used From (year)	Used Until (year)	Frequency
1.				
2.				
3.				

5) Which of the following categories would you consider your childhood diet to the age of 18. Please circle only one category unless you had a major diet shift. If so, please indicate the ages at which you followed each diet.
Meat Eater **Vegetarian** **Vegan**

6) What locations have you lived in, starting with birth and extending to age 18? Please be as specific as possible. If you require more room, please use the back of this sheet.

	From (year)	To (year)	City	State	Country
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					

7) Please indicate the approximate location of each of the above areas on the attached map. For area (1) write a ①, area (2) write a ②, etc. If you lived outside of the U.S. for any portion of your childhood, please disregard for the extent of your domicile outside of the U.S. Do include the numbers for any corresponding time lived in the U.S.

8) Have you undergone any prior dental extractions at this facility within the past year? (circle one) **Yes** **No**

If yes, please indicate the date to the best of your recollection. (day/month/year) ______________

Approved UF-IRB-01 474-2005

Figure 4-1. Joint POW/MIA Accounting Command survey.

Figure 4-1. Continued.

The aim of the survey was to control for as many sources of error or variation as possible in the data as well as create an isotopic mapping capability for natal region. All questions, with the exception of the prior dental surgery question, were pertinent to the study in that they account for factors that may possibly lead to differential maturation in teeth or absorption/deposition of the various isotopes being studied.

The first item, date of birth, allowed for temporal comparison of specimens between JPAC and VA samples versus USAFA samples. While dental development is relatively genetically conservative, there is some minor variation in dental development rates between sexes and major ethnic groupings. This information; date of birth, sex, and self-perceived race; served as potential blocking factors during data analysis.

The effects tobacco use upon isotope analyses for teeth, have thus far not been addressed in the literature. While enamel isotopic fates are locked in after amelogenesis terminates, it is unknown whether tobacco use may trigger diagenetic changes within teeth that may affect isotope values. It is commonly known to stain teeth, and may need to be accounted for in preparation protocols and in interpretation of results.

Dates and locations of childhood residence were critical for making sense of the oxygen, strontium, and lead stable isotope results. The validity of the residence information was confirmed by individuals visually approximating these areas on a map. It was also useful if someone could not remember the exact name of a town/city in which they lived but did know approximately where it was in relation to neighboring areas.

The other questions served to control for potentially confounding variables. Dental extraction history was necessary to prevent counting one individual who underwent

multiple extractions over multiple days as more than one subject. Survey questions were limited to one page with the map encompassing a second page.

Pertinent information corresponding to each patient was also recorded by the dental staff on each survey. Here, each facility assigned a unique subject identifier number to each patient (i.e., VA-001). Additionally, the position in the arcade that each tooth came from (tooth number) was noted according to the Universal/National System dental numbering scheme for permanent dentition as well as the date of extraction.

Teeth were extracted following standard dental protocols for each facility. Care was taken to preserve as much of the crown as possible. Each tooth was placed into its own vial, which was labeled with the subject identifier number and tooth number, All vials from a particular individual were then placed in a resealable bag and the bag stapled to the associated survey. The surveys and teeth from USAFA were shipped via FedEx to the C.A. Pound Human Identification Laboratory (CAPHIL). Surveys and teeth from the VA were picked up weekly and CPRS updated by the author. Teeth provided by both facilities were not stored in any solution or fixative.

Sampling

Teeth were selected for sampling using the following hierarchy. Those teeth whose cessation of amelogenesis was most similarly timed with the third molars were preferred, with other teeth chosen on a decreasing sliding scale (Table 4-1). The younger in an individual's life crown completion occurred for a specific tooth, the less desirable the tooth was for sampling. Additionally, molars were preferable as they have the largest surface area available for enamel removal. As a matter of course, mandibular teeth were chosen over maxillary teeth and right over left. Furthermore, for the East Asian reference samples from the CIL, teeth still present in the alveoli were selected over loose teeth,

Table 4-1. Crown formation/tooth eruption.

Tooth	Crown Initiation[1] (upper/lower) in yrs	Crown Completion[2] (upper/lower) in yrs	Tooth Eruption[3] (upper/lower) in yrs
3rd molar	7.0–10.0/7.0–10.0	13.3/13.3	17–21/17–21
2nd molar	2.5–3.0/2.5–3.0	6.7/6.7	12–13/11–13
2nd premolar	2.0–2.5/2.0–2.5	6.3/6.3	10–12/11–12
1st premolar	1.5–2.0/1.5–2.0	5.8/5.6	10–11/10–12
Canine	0.3–0.4/0.3–0.4	4.9/4.8	11–12/9–10
2nd incisor	0.8–1.0/0.25–0.3	4.0/4.0	8–9/7–8
1st incisor	0.25–0.3/0.25–0.3	3.7/3.6	7–8/6–7
1st molar	0.0/0.0	3.8/3.7	6–7/6–7

[1] range in Shour & Massler (1940)
[2] mean values in Anderson et al. (1976)
[3] range in ADA (1999)

since the actual tooth number could be verified more easily with the presence of the associated bone. In nearly all CIL cases however, a full arcade was not present to choose from, thus the best option according to the aforementioned sampling scheme was selected based on the resources available.

Each individual was assigned a unique identifier, not each tooth. Therefore, if two teeth were utilized from the same individual, the samples would be given the same identifier, with an additional tooth number identifier. This numbering scheme prevented inflation of actual individual numbers. Furthermore, due to potential intertooth variation in stable isotope values, enamel was not combined from multiple teeth to achieve the desired weight of enamel powder.

Central Identification Laboratory

The CIL is an American Society of Crime Laboratory Directors (ASCLD)-certified crime lab. As a result, all sampling conducted at the CIL conformed to their standard operating procedures, to ensure compliance with ASCLD requirements. Prior to

sampling, potential specimens were researched utilizing a list of “Mongoloid holds” provided by Dr. Andrew Tyrrell, the Casualty Automated Recovery and Identification System (CARIS) database, and a thorough personal investigation of the entire evidence storage area. Once suitable specimens had been identified, individual accessions were checked out from the evidence manager and transferred to the CIL autopsy suite for the actual sampling.

Study identifiers with a “CIL” prefix and a three-digit suffix were associated with lab accession numbers from the CIL Mongoloid hold collection. Two teeth, if available, were selected from each accession following the above procedures. In all cases, at least one intact and undisturbed tooth was left with the case in the event that future identification efforts, such as DNA sequencing, were required. Each tooth selected for sampling was assigned an additional sample number (01A or 02A) mirroring the lab’s DNA sampling procedures. Information cards for each tooth were created for photo cataloging and provided the following information (Figure 4-2):

- CIL accession number
- Individual designator (if applicable)
- Tooth number
- Sample identifier
- Date
- “Isotope study”
- Subject identifier number
- Researcher’s initials

From 14 June 2005 to 06 July 2005, a total of 112 teeth were sampled from 61 individuals believed to have originated from or been recovered from the following areas: Vietnam (48 individuals); Cambodia (4 individuals); Laos (3 individuals); the Korean

Figure 4-2. Pre-drilling photo of CIL-033 #19 with data card. Note: the accession number is purposely, partially obscured.

peninsula (3 individuals); the Solomon Islands (2 individuals); and the Philippines (1 individual). Teeth were eased out of their respective alveoli by hand or drilled out, when necessary, using an NSK UM50 TM slow-speed dental drill with either a #2 or #4 carbide dental drill bur, taking care to minimize damage to each alveolus. A photo, to include an information card, reference scale (ruler), and empty collection vial was taken of each tooth to document what each element looked like prior to drilling (Figure 4-2). Separate photos of the buccal or lingual and occlusal surfaces were taken. (In cases where the teeth had to be drilled out of their respective alveoli, pictures of the unaltered arcades or portions thereof were taken using the same format.)

Each tooth was then placed into a vial of 3%, household-use hydrogen peroxide and cleaned via a Branson Bransonic® 2510 tabletop ultrasonic cleaner for 30 minutes. When finished, teeth were removed from the solution and manually cleaned with a toothbrush. The enamel surface of the teeth was prepared for drilling by cleaning off excess calculus, soil, and/or staining using the same apparatus and a #8 carbide dental drill bur.

Samples of approximately 100 mg of pristine enamel were drilled off of each tooth using the same set-up (see Appendix B for drilling data). Care was taken not to drill into the dentine. Enamel powder was collected on creased weighing paper and transferred to labeled 1.5 mL microcentrifuge tubes. The drilled tooth, collection vial, scale (ruler), and information card were again photographed to document the end-stage condition of the tooth and for chain of custody purposes. The teeth were then returned to their original storage bag along with the information cards with the associated elements for that particular accession number. The bag was resealed with evidence tape, and the tape initialed and dated on both sides. The remains were then turned in to the evidence manager. Drill burs and weighing paper were discarded after each use and the drill cleaned of adherent enamel powder.

Chain of custody forms were completed for all specimens, transferring possession of the enamel powder and any associated enamel chips to the author (Appendix B). The microcentrifuge tubes containing the enamel specimens were then transported from CIL to CAPHIL through the services of FedEx.

The author also attempted to gain access to human teeth from native populations while performing duty-related activities in Vietnam from July and into August 2005. Such efforts were abandoned however, when provincial officials stated that regional and higher government officials would be required to approve any request to procure human samples.

United States Air Force Academy and Veterans Affairs

The Air Force Academy collected surveys and a total of 948 teeth from 274 individuals between late August 2005 and late April 2006 (Table 4-2; see also Appendix C for a list of survey results). Of these, one third molar was selected from each

Table 4-2. Isotope sampling matrix.

	#	#	Total # Individuals Run				Total # Teeth Run				Total # Runs			
Source	Inds	Teeth	C	O	Sr	Pb	C	O	Sr	Pb	C	O	Sr	Pb
CIL	61	112	61	61	36	36	64	64	36	36	65	65	36	42
USAFA	274	948	230	230	36	36	238	238	36	36	279	279	36	36
Total	335	1060	291	291	72	72	302	302	72	72	344	344	72	78

of 228 individuals for inclusion in the primary study. One tooth each from two individuals of unknown natal region were utilized for additional testing examining the necessity of using acetic acid to process the enamel. Samples originating from three different individuals were not used because of experimenter error in labeling the samples and erroneous or missing information provided by the subject. Furthermore, after sample AFA-185 from USAFA, samples were selectively chosen to fill in the geographic gaps until optimally, each state had a minimum of five individuals represented. This approach was chosen to reduce costs. Additionally, individuals from duplicate cities or those people born prior to 1980 were sampled as well. Collection of specimens from the VA began in mid-February 2006 and is ongoing. Unfortunately, because of the low number of teeth provided by the facility and the poor condition of these teeth (i.e., little to no enamel present) no samples were run for the current study. Sample collection is still ongoing though, with the hope that the teeth can be used at a later date.

Upon receipt at CAPHIL, teeth were soaked in 3% hydrogen peroxide in their original vials for 2 days. Teeth were then rinsed of the hydrogen peroxide with tap water and scrubbed with a toothbrush to remove surface contaminants, such as blood. Any adherent periodontal tissue or accessible neurovascular bundles were also removed. Clean teeth were allowed to air dry overnight in a ventilation hood and each tooth was stored in a separate, clean, labeled, resealable, plastic bag.

All USAFA samples contained at least one third molar, with the majority of individuals providing all four. Only third molars were run from this facility. Whole teeth, in the best overall condition were preferentially selected for drilling. If multiple teeth from an individual were of the same quality, sampling selection was based on the same criteria as mentioned for the CIL samples: mandibular teeth were chosen over maxillary teeth and right over left. Teeth exhibiting unusual crown anomalies or staining patterns and/or teeth in which the author disagreed with the dental staff numbering were photographed prior to drilling only. The remaining teeth were not photo-documented. Photo content consisted of the tooth, subject identifier number, tooth number, and a scale (Figure 4-3). Two photos, one of the buccal or lingual surface, and one of the occlusal surface were taken. Teeth were cleaned in distilled water within individual capped vials with a Branson Bransonic® 1510 tabletop ultrasonic cleaner for 30 minutes. After air-drying, teeth were cleaned of any surface contaminants to include alveolar bone remnants using a NSK UM50 TM slow-speed dental drill with a #8 carbide dental drill bur. Samples of approximately 100-200 mg of pristine enamel were drilled off of each tooth using the same set-up. Care was taken not to drill into the dentine. Enamel powder was collected on creased weighing paper and transferred to labeled 1.5 mL microcentrifuge tubes. Drill bits, weighing paper, and latex gloves were discarded after drilling each tooth and the drill cleaned of adherent enamel powder.

Figure 4-3. Pre-drilling photo of AFA-093 #32.

Carbon and Oxygen Sample Preparation

Central Identification Laboratory Samples

Chemical preparation of the enamel powder was performed in the stable isotope laboratory at the Florida Museum of Natural History, Gainesville, FL, according to the protocol developed by Dr. Pennilynn Higgins, museum postdoctoral fellow. The powder of one tooth from each individual was selected based on the integrity of the sample (i.e., whether there was the possibility of dentin or other contaminants mixed in with the enamel) and greatest mass of powder available for analysis. Organic residues were removed from the sample powder by adding 1 mL 30% hydrogen peroxide (H_2O_2) to each microcentrifuge tube. Tubes were shaken utilizing a Thermolyne Maxi-Mix 1, 16700 mixer and the lids lifted up to prevent gas pressure build-up inside the tubes. The opened vials were stored in a closed reaction cabinet. Samples were periodically shaken with the mixer, every 1 to 2 days, to re-suspend the enamel powder that had settled at the bottom of the vial.

On a weekly basis, the H_2O_2 was removed by centrifuging samples for 20 minutes at 10,000 RPM in an Eppendorf 5415D microcentrifuge and pipetting off the H_2O_2.

Pipette tips were discarded between each sample to prevent cross-contamination. Fresh H_2O_2 was then added following the same protocol. Samples were reshaken, lids opened, and placed back in the reaction cabinet. The absence of escaping air bubbles from the solution usually indicates the sample powder is finished reacting and ready for the next phase of treatment. After consulting with Drs. Bruce MacFadden, Florida Museum of Natural History, and John Krigbaum, University of Florida Department of Anthropology, the samples were decanted of all H_2O_2 after 51 days in solution, even though nearly half still appeared to be reacting. This was likely due to the large quantity of powder being processed, with most enamel samples measuring 100 mg or greater. Samples were then twice rinsed with 1 mL deionized water utilizing the same procedure for removing H_2O_2 (i.e., water added, then tubes shaken, centrifuged down, and decanted).

After rinsing the samples with deionized water and decanting all water from them, secondary carbonates were removed via an acetic acid bath. Rinsed samples, free from water, were bathed in 1 mL 0.1 N acetic acid, shaken, and allowed to sit for 30 minutes. The acetic acid was pipetted off after centrifuging the microcentrifuge tubes at 10,000 RPM for 5 minutes. Samples were then twice rinsed with deionized water and the water removed in the same manner as previously discussed. Samples were allowed to air-dry in their open microcentrifuge tubes inside of a desiccator for 2 weeks. This ensured all liquid had evaporated from the enamel powder.

An additional side test examining the necessity of performing the acetic acid step was performed. Theoretically, teeth extracted from living subjects should not have to undergo the acetic acid bath, because teeth in the living are not subject to diagenetic changes associated with the build-up of secondary carbonates due to taphonomic factors.

The acetic acid bath was performed on all samples because there is no know precedence to do without acetic acid for forensic isotope purposes. Eight USAFA and two CIL samples were split in half with one half undergoing the full protocol previously outlined and the second sample of each pair undergoing the H_2O_2 bath and rinses only. Values were then compared to determine if this step of the protocol is indeed required.

Portions of the dried enamel measuring between 1.2 mg and 1.5 mg were then loaded into stainless steel boats at the University of Florida, Department of Geological Sciences, Light Isotope Laboratory. Each boat was placed into 1 of 44 numbered slots on a brass tray (Figure 4-4) and the tray placed into a desiccator until they were run on the laboratory's VG/Micromass (now GV Instruments) PRISM Series II isotope ratio mass spectrometer with an Isocarb common acid bath preparation device. Load sheets were accomplished for each tray listing the sample name and weight for each position in the tray (Appendix D). All samples were loaded by the author. The PRISM was operated by Dr. Jason Curtis and Kathy Curtis.

The first run was organized as follows: slots 1–4, standards of NBS-19 measuring between 60 μg and 120 μg; slots 5–20, alternating enamel powder and blank positions;

Figure 4-4. Loaded tray for PRISM mass spectrometer analysis.

slots 21–22, NBS-19 standard; slots 23–42, alternating enamel powder and blank positions; slots 43–44, NBS-19 standard. This arrangement allowed for the analysis of 18 samples. Empty or blank positions were included in the first run to check for contaminants within the samples. Mass spectrometer readings for the blank positions, indicate leaching of slow-reacting sample into these positions and hence likely contamination. Contamination tends to be much more of an issue with fossilized samples versus modern or historical (Koch *et a*l 1997). Because the first run ran clean with no indication of contamination, the blanks were replaced with sample for all subsequent runs. The sample line-up for all subsequent runs therefore was as follows: slots 1–4, NBS-19 standard; slots 5–20, sample; slots 21–22, NBS-19 standard; slots 23–42, sample; slots 43–44, NBS-19 standard. This arrangement allowed for the analysis of 36 samples for each run of the mass spectrometer.

United States Air Force Academy Samples

The Academy samples were prepared in the same manner as the CIL samples with three exceptions. The first change to the processing protocol entailed reducing H_2O_2 exposure time to 24 hours. This change was made upon the recommendation of Dr. Bruce MacFadden, Florida Museum of Natural History, and Dr. Pennilynn Higgins, Stable Isotope Ratios in the Environment Analytical Laboratory, Department of Earth and Environmental Sciences, University of Rochester. It was implemented because whole teeth were cleaned for 2–3 days using a 3% H_2O_2 solution to remove external organics, but more importantly, because teeth were collected directly from living subjects. Organics associated with diagenetic transfer due to burial were not encountered with these samples as they were with the CIL samples.

Additionally, a GCA Corporation mechanical convection oven was procured at the beginning of the Academy sample preparation process. Instead of air-drying the samples in a desiccator, chemically processed samples were placed in a covered microcentrifuge tray with their tops open, and allowed to evaporate to dryness over the course of 4 days. The oven was set on a setting of "3," which equated to a circulating temperature of 52^{O} C as measured by an Ever Ready Thermometer Co., Inc. oven thermometer.

Samples were loaded for analysis by the PRISM for a 36 sample run in the aforementioned format, except enamel weight was reduced to between 1.0 mg and 1.2 mg. Larger sample size was found to slow machine processing time because excess gas produced by the larger weights required multiple rounds of evacuation from the system before readings could be made (Dr. Jason Curtis, personal communication).

Strontium and Lead Sample Preparation

Strontium and lead isotope ratios were measured for 72 total individuals, evenly split between the CIL and USAFA samples. Additional sampling was not performed due to the financial constraints of this project. Individual CIL samples were chosen based on the purity of sample and highest overall powder weight. All samples believed to originate from outside of Vietnam were sampled."

For the USAFA samples, the author and Dr. George Kamenov constructed a crude geologic regional map based on underlying basement rocks. All individuals residing in a foreign country or outside of the CONUS for the entirety or majority of amelogenesis were sampled. The remainder of the USAFA samples was drawn from a list composed of individuals who had resided only in one city for the entirety of amelogenesis. The list was split into regions and individuals chosen based on sample purity and highest powder weights. Additionally, multiple individuals from Arizona, California, and Colorado, and

Florida were selected to determine if any obvious trends emerged for strontium and lead values within the states. Sampling was completed prior to receipt of all of the USAFA samples in order to comply with program deadlines.

Sample preparation and column chemistry to extract strontium and lead from the CIL and USAFA samples was the same with one exception: the CIL samples run were those that underwent the carbon/oxygen pretreatment process, while the USAFA samples were untreated. Prior to pretreatment of the USAFA samples for carbon/oxygen stable isotope analyses, the pristine enamel power was split, with approximately 100 mg of the enamel powder being transferred to a second clean, labeled vial. This second, 100 mg portion of enamel powder was what was utilized for strontium and lead isotope ratio analysis.

All column chemistry was performed in a class-1000 rated clean lab facility within the Department of Geological Sciences, University of Florida. Strontium and lead extraction was a 5-day process once all materials had been cleaned (for cleaning procedures, Appendix E). For the CIL samples, a maximum of 12 samples could be processed during the 5-day cycle. An additional 6 lead columns were located and tested prior to running the USAFA samples, raising the number of American samples processed during the 5-day cycle to 18.

Day 1: Clean 6 mL Teflon vials were refluxed utilizing approximately 1.5 mL 6N hydrochloric acid (HCl). Vials were tightly capped and placed on a hot plate set at “3” overnight.

Day 2: Refluxed vials were triple rinsed of the HCl utilizing 4-times distilled water (4X DI H_2O) and labeled with the subject identifier number. Each microcentrifuge tube

containing enamel powder was wiped externally with a Kimwipe prior to enamel transfer. One drop of 4X DI H_2O was placed in each Teflon vial to reduce static migration of the powder. A clean microspatula was used to loosen the enamel powder in the microcentrifuge tube and the entire contents of the mircocentrifuge tube were poured into the Teflon vials. The microspatula was cleaned between each transfer utilizing 4X DI H_2O and a clean, lint-free wipe. The enamel powder within each Teflon vial was dissolved in 3 mL 50% nitric acid (HNO_3) (optima). The vials were then capped and placed overnight on the hotplate set at "3." An additional set of vials was refluxed utilizing the procedures listed in the "Day 1" protocol.

Additionally, 6 CIL samples were spiked to determine both strontium and lead concentrations. Prior to spiking, the enamel powder for each sample was partitioned into thirds and each quantity of powder was placed into a dry, refluxed Teflon vial. Water was not utilized to reduce static migration of the powder, because precise mass values for each solution were required to calculate the concentration of the heavy isotopes. An RS95A $^{85}Rb/^{84}Sr$ spike was added to 1/3 of the enamel powder to measure strontium concentration. A UF-1A ^{208}Pb spike was added to a second third of the enamel powder to determine the lead concentration. In order for the lead concentration to be calculated however, a third unspiked portion of enamel powder had to be run as well for comparative purposes. The spiked and unspiked, enamel powder counterparts were dissolved in 3 mL 50% HNO_3 (optima) and all subsequent steps followed the normal extraction protocol.

Day 3: The additional set of refluxed vials was triple rinsed of the HCl utilizing 4X DI H_2O and labeled with the subject identifier number. Back-up reserves of the samples

were then created by transferring 1 mL of the dissolved powder to the new vials. The original and back-up samples were then allowed to evaporate with the caps off on the hotplate until the samples were dry (usually 3–4 hours).

Day 4 (lead extraction): The lead fraction was separated from the dissolved tooth enamel following a protocol modified after Manhes et al. (1978). A new set of vials was set to reflux utilizing the procedures listed in the "Day 1" protocol. Short, filtered Teflon columns packed in 6N HCl acid were shaken dry and twice rinsed with 4X DI H_2O. The stem of the column and a small portion of the chamber were then filled with 4X DI H_2O, taking care to avoid introducing air bubbles into the column. Dowex 1X-8 lead resin was shaken, poured into the column chamber, and allowed to filter through until the stem of the column was packed with the resin (approximately 100µL of resin), creating a "resin bed." Excess resin was pipetted out. The resin in each column was then washed with 2 mL 6N HCl (optima grade).

While the acid was filtering through the columns, the original, evaporated samples were dissolved in 300 µL 1N hydrogen bromide acid (HBr) (seastar), capped, and placed on a hotplate set at "3" for approximately 4 minutes. Dissolved samples were removed from the hotplate and set aside until needed. Once all of the acid had passed through the column, 200 µL of the dissolved sample was loaded into the column. The collection beaker under the column was exchanged for the original vial to collect the sample for the next day's strontium collection. Sample numbers were written on paper towels in front of each column to prevent confusion.

Once the sample had filtered through, the resins were bathed in three subsequent washes of 1 mL 1N HBr (seastar). After the second wash, the original sample vials were

exchanged for the previously used collection beakers and the vials were placed on a hotplate set at “3” until completely evaporated. During completion of the third wash, the refluxed vials were triple rinsed of the HCl utilizing 4X DI H_2O and labeled with the subject identifier number. After the third wash, the collection beakers were switched out for the newly-refluxed, labeled, final collection vials. The lead fraction was collected utilizing 1 mL 20% HNO_3 (optima grade). The vials containing the lead solution were then evaporated to dryness on a hotplate set at “3.” The columns were cleaned using a flushing bottle of 4X DI H_2O and placed in a jar of 6N HCl for a minimum of 12 hours.

Day 5 (strontium extraction): The strontium fraction was separated out following the strontium-specific procedure designed by Pin and Bassin (1992). A new set of vials was set to reflux utilizing the procedures listed in the “Day 1” protocol. Long, filtered Teflon columns packed in 6 N HCl acid were shaken dry and twice rinsed with 4X DI H_2O. The stem of the column and a small portion of the chamber were then filled with 4X DI H_2O, taking care to avoid introducing air bubbles into the column. EI Chrom Part #-B100-S strontium resin was shaken, poured into the column chamber, and allowed to filter through until the stem of the column was packed with the resin (approximately 100µL of resin), creating a “resin bed.” Excess resin was pipetted out. The resin in each column was then equilibrated with 2 mL 3.5N HNO_3.

While the acid was filtering through the columns, the dried samples were dissolved in 350 µL 3.5N HNO_3. Once all of the acid had passed through the column, 100 µL of the dissolved sample was loaded into the column. Sample numbers were written on paper towels in front of each column to prevent confusion. Once the sample had filtered through, the resins were bathed in four subsequent washes of 100 µL 3.5N HNO_3. A

final wash of 1 mL 3.5N HNO_3 was performed prior to collection. During completion of the final wash, the refluxed vials were triple rinsed of HCl utilizing 4X DI H_2O and labeled with the subject identifier number. After completion of this step, the collection beakers were switched out for the newly-refluxed, labeled, final collection vials. The strontium fraction was collected by rinsing the columns in 1.5 mL 4X DI H_2O. The vials containing the strontium solution were then evaporated to dryness on a hotplate set at "3." The columns were cleaned using a flushing bottle of 4X DI H_2O and placed in a jar of 6N HCl for a minimum of 12 hours.

Mass spectrometry: Both the lead and strontium fractions were analyzed utilizing the University of Florida, Department of Geological Science's, Nu-Plasma, Multi-Collector, Inductively-Coupled Plasma Mass Spectrometer (MC-ICP-MS). Evaporated samples were dissolved in 500 µL 2% HNO_3 (optima). Initial analyses for both lead and strontium utilized 50 µL of dissolved sample and an additional 950 µL 2% HNO_3. Ratios of sample to 2% HNO_3 were then adjusted accordingly so the voltage read by the MC-ICP-MS for each sample fell ideally between 2.0 and 8.0 volts.

For mass-bias correction, lead solutions were spiked with thallium just before the analyses in order to avoid thallium oxidation (Kamenov et al. 2004). Sample and standard solutions were aspirated either through a Nu Instruments desolvating nebulizer (DSN-100) ("dry plasma" mode) or directly into the plasma source through a Micromist nebulizer with GE spray chamber ("wet plasma" mode). Measured uptake rate for both sample introduction methods was about 100µl min^{-1}. The instrument settings were carefully tuned to maximize the signal intensities on a daily basis (Kamenov personal communication). Preamplifier gain calibrations were run before the beginning of each

analytical session. The analyses reported here were conducted in static Time-Resolved Analysis (TRA) mode. All lead isotope ratios are relative to the following values for NBS 981: $^{206}Pb/^{204}Pb$ = 16.937 (+/-0.004 2σ), $^{207}Pb/^{204}Pb$ = 15.490 (+/-0.003 2σ), and $^{208}Pb/^{204}Pb$ = 36.695 (+/-0.009 2σ) (Kamenov et al. 2005).

According to Dr. George Kamenov, strontium isotopic ratios were acquired in static mode using 5 Faraday collectors (personal communication). $^{87}Sr/^{86}Sr$ was corrected for mass-bias using exponential law and $^{86}Sr/^{88}Sr$=0.1194. ^{87}Sr was corrected for presence of rubidium by monitoring the intensity of ^{85}Rb and subtracting the intensity of ^{87}Rb from the intensity of ^{87}Sr, using $^{87}Rb/^{85}Rb$=0.386. All analyses were done in TRA mode by using previous measured zeros determined on clean 2% HNO_3 solution in order to correct for isobaric interferences from the presence of impurities of krypton in the argon gas. Analyses of the NBS 987 strontium standard during the course of this study gave $^{87}Sr/^{86}Sr$=0.71025 (+/-0.00004, 2 σ) (Dr. George Kamenov personal communication).

The machine was purged of any residual sample volume by introducing a pre-wash of 5% HNO_3 and then a wash of 2% HNO_3 through the MC-ICP-MS between each sample run. A solution of the NBS 981 standard for lead and NBS-987 for strontium, was run every six samples to ensure proper calibration of the machine. The author operated the MC-ICP-MS under the supervision of Dr. George Kamenov.

Statistical Analyses

Sample sizes of at least 30 in each population satisfied the requirement for approximate normality, the variances were assumed to be homogenous and the samples independent, and random sampling was assumed since everyone presenting for dental

extraction was queried to participate in the study (Ott and Longnecker 2004). (Note: it was realized that this was not a truly random sample because subjects utilized were only those who consented to participate). The null hypotheses that no significant differences exist for the mean values of each isotope between individuals from East Asia and the United States, nor between different regions of the United States, were tested via the general linear model (GLM) procedure in SAS version 9.1. The GLM procedure was in the form of a multivariate analysis of variance (MANOVA). This approach was chosen because of the presence of several dependent variables measured for multiple samples. The GLM function was utilized over a straight MANOVA because of unbalanced sample sizes between populations (SAS 9.1).

Exploratory data analyses included various plots to identify data trends and outliers and the calculation of summary statistics. General linear models were employed to assess the differences between population means due to the populations having different numbers of observations. When three populations were compared simultaneously, a Tukey studentized range test to control for Type I experiment-wise error was included in the analysis. Paired t-tests were performed to determine if the acetic acid step was necessary in the chemical preparation of teeth from living subjects. Linear discriminant function analyses were conducted in an attempt to create an equation that would correctly classify an individual as either being of Southeast Asian origin or of American, based on the isotopes studied.

In an attempt to identify potentially confounding variables addressed in the survey such as age, sex, race, tobacco use, and childhood diet, the general linear model was also performed to assess the differences between variable means. When greater than two

subsets of a particular variable were compared, a Tukey-Kramer adjustment for multiple comparisons was included to control for Type I error. A discriminant function analysis was also accomplished to determine if an equation could be created utilizing the isotopes studies that would successfully categorize an individual as originating from a particular region within the United States. In addition, a test for correlation was conducted to determine the relationship between the $\delta^{18}O$ values and the corresponding latitudinal coordinates of the natal regions of the donors.

CHAPTER 5
ANALYTICAL COMPARISION OF EAST ASIAN AND AMERICAN SAMPLES

The first question this project sought to answer was whether the isotopic composition of enamel could be differentiated between East Asians and Americans. Significant differences were found between the least squares means of the Central Identification Laboratory (CIL) and United States Air Force Academy (USAFA) samples for all isotope values examined (Table 5-1), demonstrating that all isotopes examined are potentially useful in distinguishing between these two populations.

Light Isotopes

The results from the PRISM analysis are presented in Table 5-2 and represented in Figure 5-1. Location was assessed as believed provenance for the CIL samples and locations of residence during amelogenesis of the third molar (ages 7-18 [Fanning & Brown 1971]) for the USAFA samples. Precision, as determined by replicate analyses of 86 separate NBS 19 standards over 13 machine-days from 01 December 2005 through 01 June 2006, was measured at 0.08‰ for carbon and 0.14‰ for oxygen. Similar numbers were calculated based on replicate measures of homogenous sample material with average standard deviations of 0.04‰ for carbon and 0.13‰ for oxygen.

Carbon

Carbon isotope ratios appear to be an excellent discriminator of natal origin between East Asian and American populations based on cultural dietary preferences. The least squares means of the two groups were significantly different, with a p-value of

Table 5-1. Summary statistics and general linear model results of all isotopes examined for CIL samples compared to USAFA samples (CIL outlier excluded). All values are in ‰.

Population	N	Variable	Min	Max	Mean	Std Dev	P-val
CIL	60	$\delta^{13}C$	-17.25	-11.60	-14.25	1.00	<0.0001
USAFA	228	$\delta^{13}C$	-12.88	-7.77	-9.97	0.81	
CIL	60	$\delta^{18}O$	-10.61	-5.04	-7.45	0.90	0.0092
USAFA	228	$\delta^{18}O$	-12.57	-3.14	-6.88	1.63	
CIL	35	$^{87}Sr/^{86}Sr$	0.706811	0.721172	0.710995	0.002938	0.0013
USAFA	36	$^{87}Sr/^{86}Sr$	0.707449	0.711186	0.709273	0.000883	
CIL	35	$^{208}Pb/^{204}Pb$	37.176100	38.883700	38.074157	0.355069	0.0073
USAFA	36	$^{208}Pb/^{204}Pb$	37.398300	38.616500	38.268931	0.225698	
CIL	35	$^{207}Pb/^{204}Pb$	15.528800	15.695800	15.603740	0.040095	0.0006
USAFA	36	$^{207}Pb/^{204}Pb$	15.556800	15.674900	15.631292	0.022565	
CIL	35	$^{206}Pb/^{204}Pb$	16.991700	19.620500	18.090809	0.435832	<0.0001
USAFA	36	$^{206}Pb/^{204}Pb$	17.681000	19.049300	18.595094	0.280822	
CIL	35	$^{208}Pb/^{206}Pb$	1.958010	2.254450	2.105517	0.040400	<0.0001
USAFA	36	$^{208}Pb/^{206}Pb$	2.027260	2.115030	2.058318	0.020901	
CIL	35	$^{207}Pb/^{206}Pb$	0.799982	0.914750	0.862969	0.018726	<0.0001
USAFA	36	$^{207}Pb/^{206}Pb$	0.822855	0.879814	0.840787	0.011796	

<0.0001. The predominance of rice in East Asian diets appears to lead to more depleted $\delta^{13}C$ values compared to young American adults who had less negative $\delta^{13}C$ values, due to a high preponderance of corn and sugar in their diet.

Table 5-2. Carbon and oxygen isotope results. All values are in ‰.

Identifier	$\delta^{13}C$	$\delta^{18}O$	Location	Identifier	$\delta^{13}C$	$\delta^{18}O$	Location
CIL-001	-13.09	-7.73	Vietnam	CIL-032	-14.58	-6.78	Cambodia
CIL-002	-13.72	-6.54	Vietnam	CIL-033	-14.79	-8.11	Cambodia
CIL-003	-15.17	-7.33	Vietnam	CIL-034	-16.17	-10.25	Cambodia
CIL-004	-14.47	-7.34	Vietnam	CIL-035	-14.01	-7.83	Vietnam
CIL-005	-14.69	-5.95	Vietnam	CIL-036	-13.45	-7.25	Vietnam
CIL-006	-13.03	-7.47	Vietnam	CIL-037	-13.45	-7.82	Cambodia
CIL-007	-13.64	-7.71	Vietnam	CIL-038	-13.74	-6.68	Vietnam
CIL-008	-14.32	-9.27	Solomon Isl.	CIL-039	-13.66	-8.50	Philippines
CIL-009	-14.43	-7.84	Vietnam	CIL-040	-15.08	-8.56	Solomon Isl.
CIL-010	-11.60	-7.76	Vietnam	CIL-041	-15.03	-7.11	Vietnam
CIL-011	-14.99	-8.23	Vietnam	CIL-042	-14.20	-6.90	Vietnam
CIL-012	-14.68	-7.24	Vietnam	CIL-043	-14.63	-6.90	Vietnam
CIL-013	-14.52	-7.75	Laos	CIL-044	-14.43	-7.69	Vietnam
CIL-014	-14.47	-7.65	Vietnam	CIL-045	-14.10	-6.83	Vietnam
CIL-015	-15.16	-7.89	Laos	CIL-046	-14.44	-7.16	Vietnam
CIL-016	-15.02	-7.97	Vietnam	CIL-047	-14.72	-6.92	Vietnam
CIL-017	-12.17	-8.07	Vietnam	CIL-048	-13.83	-8.90	Vietnam
CIL-018	-14.27	-6.96	Vietnam	CIL-049	-15.31	-7.77	Vietnam
CIL-019	-15.45	-6.52	Vietnam	CIL-050	-13.90	-7.04	Vietnam
CIL-020	-12.64	-7.40	Vietnam	CIL-051	-15.45	-6.57	Vietnam
CIL-021	-13.57	-7.42	Laos	CIL-052	-12.20	-7.88	Vietnam
CIL-022	-14.52	-8.06	Vietnam	CIL-053	-14.45	-6.60	Vietnam
CIL-023	-15.22	-7.75	Vietnam	CIL-054	-14.58	-7.41	Vietnam
CIL-024	-14.29	-7.50	Vietnam	CIL-055	-15.43	-6.22	Vietnam
CIL-025	-12.90	-7.07	Vietnam	CIL-056	-14.90	-7.28	Vietnam
CIL-026	-12.59	-7.25	Korea	CIL-057	-14.72	-6.80	Vietnam
CIL-027	-14.05	-6.95	Vietnam	CIL-058	-17.25	-10.61	Vietnam
CIL-028	-14.07	-7.07	Korea	CIL-059	-14.53	-6.64	Vietnam
CIL-029	-6.32	-8.24	Korea	CIL-060	-14.88	-6.75	Vietnam
CIL-030	-14.65	-6.94	Vietnam	CIL-061	-12.74	-7.60	Vietnam
CIL-031	-12.96	-5.04	Vietnam				

Table 5-2. Continued.

Identifier	$\delta^{13}C$	$\delta^{18}O$	Location	Identifier	$\delta^{13}C$	$\delta^{18}O$	Location
AFA-001	-10.43	-7.49	OR	AFA-044	-9.96	-8.91	MN
AFA-002	-10.17	-7.48	US/intl-mix	AFA-045	-10.49	-7.67	US-mix
AFA-003	-9.80	-4.22	US-mix	AFA-046	-10.45	-6.28	IL
AFA-004	-11.25	-6.53	TN	AFA-047	-11.47	-7.47	Philippines
AFA-005	-11.10	-9.22	NY	AFA-048	-9.05	-5.38	TX
AFA-006	-10.78	-8.27	CA	AFA-049	-9.63	-6.88	OH
AFA-007	-9.92	-7.56	US-mix	AFA-050	-10.45	-6.97	US/intl-mix
AFA-008	-9.77	-6.92	TX	AFA-051	-9.98	-10.21	CO
AFA-009	-10.45	-5.91	SC	AFA-052	-10.45	-7.39	CA
AFA-010	-9.20	-6.73	US-mix	AFA-053	-9.11	-4.49	US-mix
AFA-011	-9.29	-6.50	AR	AFA-054	-9.64	-6.85	US/intl-mix
AFA-012	-10.58	-10.52	US-mix	AFA-055	-10.30	-7.46	US-mix
AFA-013	-9.90	-8.00	NM	AFA-056	-10.63	-7.18	MN
AFA-014	-9.92	-4.98	TX	AFA-057	-9.61	-8.48	CA
AFA-016	-11.31	-5.69	US-mix	AFA-058	-9.75	-6.50	VA
AFA-017	-9.64	-7.83	AZ	AFA-059	-8.86	-6.99	WI
AFA-018	-9.88	-5.22	US-mix	AFA-060	-9.81	-8.98	NE
AFA-019	-9.13	-6.48	MA	AFA-061	-10.41	-7.57	CA
AFA-020	-9.39	-5.19	US-mix	AFA-062	-9.35	-4.71	TX
AFA-021	-9.69	-8.52	AZ	AFA-063	-11.23	-12.57	AK
AFA-022	-10.10	-3.14	US-mix	AFA-064	-11.33	-7.61	US-mix
AFA-023	-9.80	-5.98	Guam	AFA-065	-10.17	-6.79	CO
AFA-024	-10.19	-10.16	CO	AFA-066	-8.48	-6.41	IL
AFA-025	-10.40	-6.81	MI	AFA-067	-10.82	-6.99	US/intl-mix
AFA-026	-10.76	-9.02	US-mix	AFA-068	-9.51	-7.29	US/intl-mix
AFA-027	-9.32	-7.95	US-mix	AFA-069	-10.45	-5.67	NJ
AFA-028	-9.82	-6.41	TX	AFA-070	-10.16	-4.54	FL
AFA-029	-9.73	-5.75	TX	AFA-071	-9.81	-7.81	US-mix
AFA-030	-11.57	-6.38	CA	AFA-072	-10.16	-5.83	US-mix
AFA-031	-10.87	-9.52	CO	AFA-073	-10.80	-5.01	TX
AFA-032	-12.52	-12.40	US/intl-mix	AFA-074	-9.51	-5.58	AR
AFA-033	-10.36	-7.25	MA	AFA-075	-12.30	-5.47	US/intl-mix
AFA-034	-10.66	-8.69	WA	AFA-076B	-9.91	-7.52	CA
AFA-037	-10.33	-6.41	TN	AFA-077	-9.72	-4.37	US-mix
AFA-038	-10.05	-6.36	US-mix	AFA-078	-9.98	-7.28	VT
AFA-039	-9.49	-5.72	TX	AFA-079	-9.28	-8.93	US-mix
AFA-040	-9.10	-5.98	SC	AFA-080	-10.14	-6.61	OH
AFA-041	-10.65	-7.03	US/intl-mix	AFA-081	-10.65	-8.05	US-mix
AFA-042	-9.00	-5.75	US-mix	AFA-082	-9.59	-6.58	IA
AFA-043	-10.28	-5.83	GA	AFA-083	-9.94	-5.12	TX

Table 5-2. Continued.

Identifier	$\delta^{13}C$	$\delta^{18}O$	Location	Identifier	$\delta^{13}C$	$\delta^{18}O$	Location
AFA-084	-8.92	-5.23	US-mix	AFA-124	-8.33	-6.40	NE
AFA-085	-9.76	-6.74	CT	AFA-125	-10.54	-7.38	OR
AFA-086	-10.43	-5.52	HI	AFA-126	-10.32	-7.37	CA
AFA-087	-9.49	-4.90	GA	AFA-127	-9.95	-5.60	US-mix
AFA-088	-9.47	-6.34	OH	AFA-128	-9.71	-4.39	US-mix
AFA-089	-9.66	-5.20	FL	AFA-129	-8.91	-6.03	US-mix
AFA-090	-10.41	-6.46	MA	AFA-130	-9.44	-6.23	US-mix
AFA-091	-9.75	-6.02	TX	AFA-131	-9.63	-7.96	US-mix
AFA-092	-10.72	-5.33	NC	AFA-132	-11.14	-6.99	US/intl-mix
AFA-093	-10.02	-7.13	OH	AFA-133	-10.01	-5.41	AL
AFA-094	-11.08	-6.35	SC	AFA-134	-9.96	-6.09	MS
AFA-095	-8.44	-7.17	PA	AFA-135	-10.71	-5.43	US-mix
AFA-096	-9.20	-6.71	VA	AFA-136	-11.76	-11.46	ID
AFA-097	-10.38	-6.15	TX	AFA-137	-9.50	-6.10	US-mix
AFA-098	-9.34	-6.43	IA	AFA-138	-9.11	-5.45	NC
AFA-099	-8.72	-9.88	CO	AFA-139	-10.29	-4.79	US/intl-mix
AFA-100	-9.99	-7.23	US/intl-mix	AFA-140	-9.78	-6.20	US-mix
AFA-101	-9.36	-6.74	MA	AFA-141	-11.52	-6.59	US/intl-mix
AFA-102	-9.95	-6.59	NJ	AFA-142	-10.42	-5.47	US/intl-mix
AFA-103	-8.51	-10.16	CO	AFA-143	-10.58	-7.36	intl-mix
AFA-104	-8.97	-7.42	US-mix	AFA-144	-9.85	-7.97	US-mix
AFA-105	-11.02	-6.07	NJ	AFA-145	-10.38	-9.85	WY
AFA-106	-9.71	-8.54	US-mix	AFA-146	-10.02	-9.76	ID
AFA-107	-10.39	-9.03	OR	AFA-147	-9.64	-5.53	TN
AFA-108	-10.34	-7.46	OR	AFA-148	-12.44	-7.34	Korea
AFA-109	-9.42	-9.73	Peru	AFA-149	-10.30	-6.47	MO
AFA-110	-9.71	-4.51	TX	AFA-150	-9.20	-6.41	US/intl-mix
AFA-111	-10.53	-7.87	CA	AFA-151	-10.04	-4.55	TX
AFA-112	-9.63	-8.22	NM	AFA-152	-10.97	-7.45	US/intl-mix
AFA-113	-9.85	-5.36	GA	AFA-153	-9.43	-6.76	PA
AFA-114	-8.40	-4.88	US-mix	AFA-154	-10.03	-6.21	US-mix
AFA-115	-9.12	-6.42	TN	AFA-155	-9.25	-4.73	TX
AFA-116	-10.74	-8.84	CA	AFA-156	-10.88	-4.11	TX
AFA-117	-11.06	-8.51	WA	AFA-157	-9.56	-7.25	NE
AFA-118	-7.77	-6.02	GA	AFA-158	-10.33	-6.80	MD
AFA-119	-8.87	-6.87	VT	AFA-159	-10.32	-7.40	US-mix
AFA-120	-10.43	-7.14	US/intl-mix	AFA-160	-9.77	-4.13	FL
AFA-121	-10.59	-11.20	MT	AFA-161	-9.56	-8.41	MI
AFA-122	-10.51	-6.44	US-mix	AFA-162	-10.11	-6.76	US-mix
AFA-123	-10.47	-8.75	US-mix	AFA-163	-9.51	-5.36	TX

Table 5-2. Continued.

Identifier	$\delta^{13}C$	$\delta^{18}O$	Location	Identifier	$\delta^{13}C$	$\delta^{18}O$	Location
AFA-164	-8.91	-5.62	GA	AFA-205	-7.98	-4.82	GA
AFA-165	-9.41	-5.57	US-mix	AFA-211	-10.14	-9.83	UT
AFA-166	-10.07	-10.18	ND	AFA-212	-10.57	-10.71	MT
AFA-167	-9.98	-5.06	TX	AFA-217	-10.51	-5.78	TN
AFA-168	-11.09	-7.49	PA	AFA-218	-9.68	-7.20	NY
AFA-169	-8.85	-7.00	US-mix	AFA-220	-12.88	-4.61	Suriname
AFA-170	-9.83	-7.30	WI	AFA-221	-9.88	-5.85	WV
AFA-171	-9.08	-7.34	NJ	AFA-222	-9.51	-6.00	OK
AFA-172	-9.89	-4.76	TX	AFA-223	-10.04	-8.58	CA
AFA-173	-11.17	-7.71	CA	AFA-225	-8.03	-7.14	AZ
AFA-174	-9.39	-5.88	KY	AFA-226	-10.10	-11.60	MT
AFA-175	-10.49	-6.27	US-mix	AFA-227	-9.08	-5.84	SC
AFA-176	-9.40	-5.46	FL	AFA-228	-9.77	-7.49	WV
AFA-177	-9.78	-6.53	US/intl-mix	AFA-230	-9.54	-6.82	IL
AFA-178	-9.22	-5.80	US/intl-mix	AFA-231	-10.22	-6.90	MI
AFA-179	-9.70	-7.46	OH	AFA-235	-9.99	-6.28	OH
AFA-180	-9.96	-4.64	FL	AFA-237	-9.76	-7.98	SD
AFA-181	-10.17	-5.44	TX	AFA-240	-8.69	-4.96	US-mix
AFA-182	-8.47	-6.91	PA	AFA-241	-9.91	-9.13	CO
AFA-183	-10.35	-8.19	CA	AFA-246	-10.67	-9.70	MT
AFA-184	-12.69	-6.37	intl-mix	AFA-250	-9.97	-6.73	CA
AFA-186	-9.18	-4.75	OK	AFA-251	-10.03	-4.92	AL
AFA-187	-9.64	-5.85	IN	AFA-252	-11.48	-10.30	UT
AFA-188	-10.60	-7.30	MI	AFA-254	-9.56	-5.75	VA
AFA-192	-9.96	-8.26	OR	AFA-257	-8.89	-6.29	PA
AFA-194	-9.54	-9.79	CO	AFA-263	-9.73	-6.74	US/intl-mix
AFA-195	-9.88	-6.62	NJ	AFA-264	-9.01	-7.17	WI
AFA-196	-8.37	-5.09	GA	AFA-265	-9.83	-8.25	CO
AFA-197	-9.91	-6.63	MI	AFA-267	-9.95	-6.77	.
AFA-198	-10.41	-6.93	MN	AFA-270	-9.88	-9.06	CO
AFA-200	-10.04	-6.83	NM	AFA-272	-8.72	-4.35	GA
AFA-201	-10.99	-6.42	IN	AFA-273	-8.69	-5.55	MD
AFA-203	-9.25	-7.09	MN	AFA-274	-9.88	-3.75	FL
AFA-204	-10.69	-6.88	IL	AFA-276	-9.37	-5.82	NY

One extreme outlier was present in the CIL sample, representing an individual disinterred from Korea, near the de-militarized zone. While the oxygen value does not appear out of the ordinary, the carbon value of -6.32‰ for CIL-029 exceeds the range of

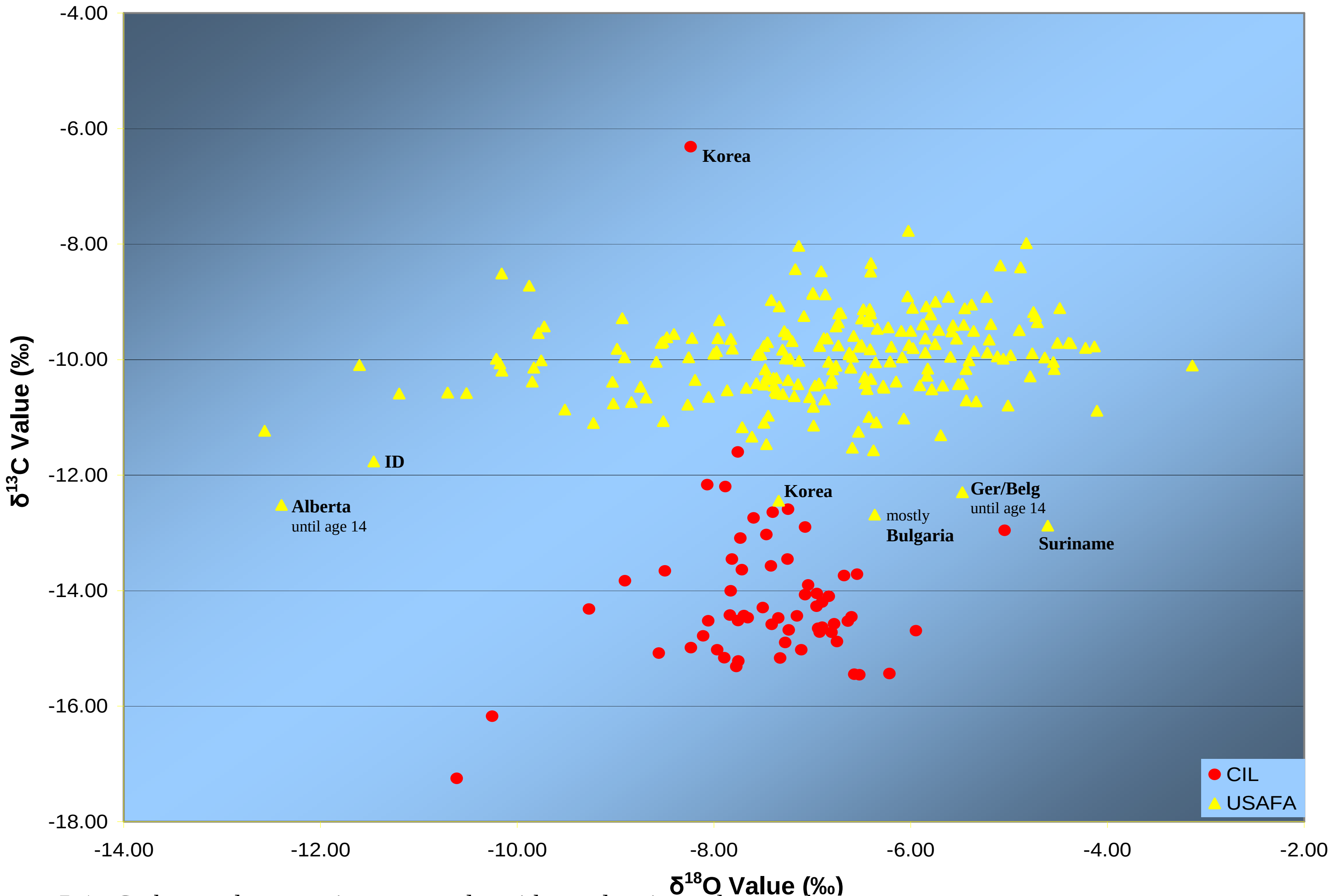

Figure 5-1. Carbon and oxygen isotope results with overlapping value overlay.

both the CIL and USAFA samples. The nearest CIL sample to this value is an individual from Vietnam, whose $\delta^{13}C$ value was measured at -11.6‰. In fact, the least negative USAFA sample was -7.77‰, still nowhere close to the outlying value. A second sample of the same tooth was rerun with a $\delta^{13}C$ of -6.20‰. This is even more enriched than the first value. While the difference of 0.12‰ for carbon exceeds the precision of the machine by 0.04‰, it is possible that additional variation arose from contaminants or through the bulk sampling process itself.

If you take the upper limit for C_4 plants at $\delta^{13}C$ = -9‰ (van der Merwe 1982) and add in a fractionation factor of +9.6‰ (DeNiro & Epstein 1978b) for bone apatite, it is apparent than an organism feeding on a monotonous diet of the most enriched C_4 plants could theoretically display a bone $\delta^{13}C$ value of approximately +0.04‰. A $\delta^{13}C$ value of -6.32‰ for apatite is not unheard of in the literature. Lee-Thorp et al. (1993) reported three human $\delta^{13}C$ values dating from the Iron Age as being near or above CIL-029, the highest of which was -5.5‰. The site was from southern Africa and the results pointed to an overwhelming dependence upon C_4 foods or livestock grazing upon C_4 grasses.

High collagen values were also found among infants from a protohistoric Amerindian village in southern Ontario, the most enriched of which was -6.8‰ (equivalent to approximately -2‰ for apatite) and attributed to a weaning diet almost exclusively of maize. Finally, such high values have also been reported in the tooth enamel of a hominid cousin, *Australopithecus africanus*. Two separate studies have measured $\delta^{13}C$ values as high as -5.6‰ (Sponheimer & Lee-Thorp 1999a) and -4.4‰ (van der Merwe et al. 2003). If CIL-029 truly was of Korean origin though, it is highly unlikely they subsisted on an exclusively C_4-based diet.

One possible explanation for this seemingly errant value is the issue of contamination. Another though, is that this person is not truly of Korean origin. The Korean War was a global conflict, with the United Nations steering the efforts to liberate the Republic of Korea from the invading communist forces from the north (Drew & Snow 2003). The United Nations command was manned by combat troops from not only the United States, but Australia, Belgium, Canada, Colombia, Ethiopia, France, Greece, Luxembourg, the Netherlands, New Zealand, the Philippines, Thailand, and Turkey. South Africa provided airpower; Denmark, India, Norway, and Sweden supplied medical units; and Italy provided a hospital. The North Koreans also had assistance in the form of foot-soldiers from China and Soviet pilots engaged the United Nations air forces (Snow & Drew 2003, Evanhoe 2006). The potential exists then for a body disinterred from an unmarked grave to be an individual from any one of these nations. In light of these factors, the isotope values for CIL-029 were not included in the statistical analyses, due to suspect provenance.

A second tooth from the same individual was drilled with $\delta^{13}C$ and $\delta^{18}O$ values of -7.63‰ and -7.50‰ respectively. These values are markedly different from the values from the first tooth (Table 5-3), but are most likely explained by intertooth variation. The raw data from Beard and Johnson (2000) indicate their range of same individual intertooth values was as large as 24.7‰ for strontium. Because different teeth mineralize at different points during childhood, each will give a slightly different still-frame value of diet due to the progression of dietary intake from breast milk to adult foods during an individual's formative years. The same can be said of geolocation as the person changes residence or is exposed to different environmental conditions during their childhood. The

Table 5-3. Central Identification Laboratory outlier run data.

Identifier	Tooth #	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	Date
CIL-029	17	-6.32	-8.24	10 Jan 06
CIL-029	17	-6.20	-8.18	5 Feb 06
CIL-029	18	-7.63	-7.50	1 May 06

period of amelogenesis of tooth #17 is from ages 7-18 in Caucasians while the period of amelogenesis of tooth #18 is roughly from ages 3-8 in Caucasians (Fanning & Brown 1971). This should be applicable for Asian teeth as well, since variation at age of amelogenesis only varies by a few months between populations (Hillson 1996).

When the CIL outlier is excluded from comparison, only six USAFA values overlapped with the main cluster of East Asian values (Table 5-4), the most enriched of which measured -11.60‰. It is interesting to note that all but one of these values correspond to individuals reared primarily outside of the United States. The most depleted $\delta^{13}C$ USAFA value was from an individual reared in Suriname. Of the remaining overlapping USAFA values, the individuals originated from primarily Bulgaria (AFA-184); Alberta, Canada until age 14 (AFA-032); Korea (AFA-148); Germany and Belgium until age 14 (AFA-075); and a Caucasian male from Idaho (AFA-136). The next two closest values are within 0.03‰ and 0.08‰ but do not overlap. They respectively belong to a self-reported Filipino raised in California and a self-reported Asian who was raised primarily abroad in Korea, Germany, and England with one 3-year tour in New York. Only one out of the next four closest values belonged to an Asian individual, who was raised in the Philippines, while the other three values are all associated with Caucasians raised in various locations throughout the United States.

To examine how individuals reared outside the continental United States (CONUS, which excludes Alaska) influenced the USAFA-CIL delta value comparisons, three

Table 5-4. $\delta^{13}C$ value comparison. Twelve most enriched CIL samples and 12 most depleted USAFA samples (CIL outlier excluded). All values measured in ‰.

	East Asian				USAFA	
Identifier	$\delta^{13}C$ (‰)	Location		Identifier	$\delta^{13}C$(‰)	Location
CIL-010	-11.60	Vietnam	↑	AFA-220	-12.88	Suriname
CIL-017	-12.17	Vietnam		AFA-184	-12.69	intl-mix
CIL-052	-12.20	Vietnam		AFA-032	-12.52	US/intl-mix
CIL-026	-12.59	Korea		AFA-148	-12.44	Korea
CIL-020	-12.64	Vietnam		AFA-075	-12.30	US/intl-mix
CIL-061	-12.74	Vietnam	Overlap	AFA-136	-11.76	ID
CIL-025	-12.90	Vietnam		AFA-030	-11.57	CA
CIL-031	-12.96	Vietnam		AFA-141	-11.52	US/intl-mix
CIL-006	-13.03	Vietnam		AFA-252	-11.48	UT
CIL-001	-13.09	Vietnam		AFA-047	-11.47	Philippines
CIL-036	-13.45	Vietnam		AFA-064	-11.33	US-mix
CIL-037	-13.45	Laos		AFA-016	-11.31	US-mix

iterations of the general linear model procedure were run. The first was the standard comparison between all CIL samples minus the outlier and all USAFA samples, which found the $\delta^{13}C$ means were significantly different at a p-value of <0.0001 (Table 5-1 and Figure 5-1). A second statistical run weighed the CIL values against not only USAFA samples from individuals residing for at least a portion of amelogenesis within the United States and its territories but against those solely raised in a foreign locale (two mixed international locations, and 1 each Suriname, Peru, Korea, and the Philippines) and among the two USAFA populations (Figure 5-2). The means for the three groups were compared and a Tukey's studentized range test performed to control for Type I experiment-wise error rate. Results indicate that the means for carbon for all three groups are significantly different at $\alpha=0.05$ (Table 5-5).

One last iteration compared the CIL samples, USAFA individuals spending at least a portion of their natal residence within the CONUS, and those USAFA samples arising from donors who were raised outside of the CONUS. Statistics were computed in

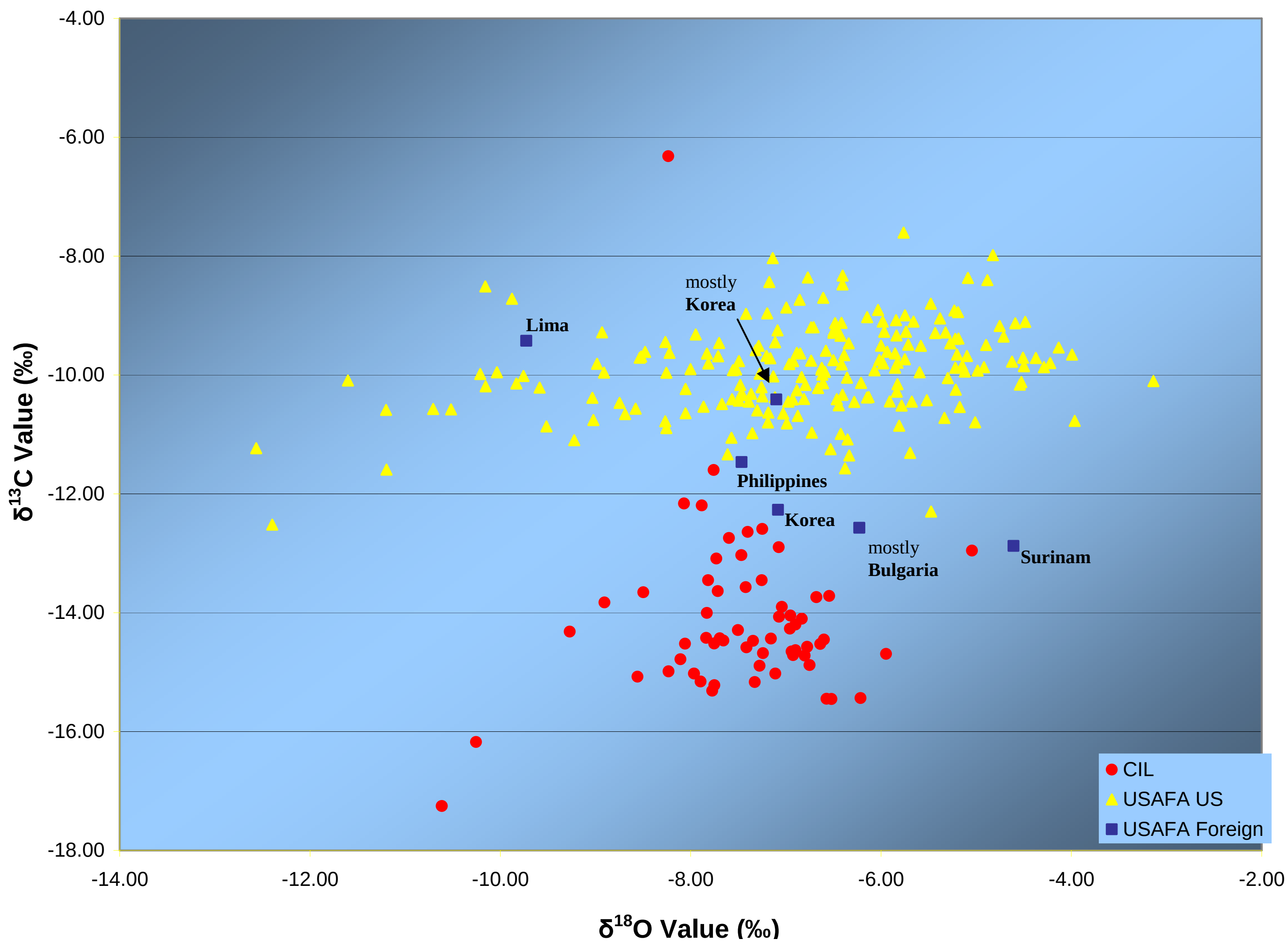

Figure 5-2. Carbon and oxygen isotope results for American and foreign USAFA comparison.

Table 5-5. Summary statistics and general linear model results of all isotopes examined for American and foreign USAFA comparison (CIL outlier excluded). All values are in ‰.

Population	N	Variable	Min	Max	Mean	Std Dev	
CIL	60	$\delta^{13}C$	-17.25	-11.60	-14.25	1.00	*†
USAFA (US)	222	$\delta^{13}C$	-12.52	-7.77	-9.92	0.75	* ‡
USAFA (foreign)	6	$\delta^{13}C$	-12.88	-9.42	-11.58	1.37	†‡
CIL	60	$\delta^{18}O$	-10.61	-5.04	-7.45	0.90	*
USAFA (US)	222	$\delta^{18}O$	-12.57	-3.14	-6.87	1.63	*
USAFA (foreign)	6	$\delta^{18}O$	-9.73	-4.61	-7.17	1.67	

*, †, or ‡ indicates significant difference between means

Table 5-6. Summary statistics and general linear model results of all isotopes examined for CONUS and overseas USAFA comparison (CIL outlier excluded). All values are in ‰.

Population	N	Variable	Min	Max	Mean	Std Dev	
CIL	60	$\delta^{13}C$	-17.25	-11.60	-14.25	1.00	*†
USAFA (CONUS)	219	$\delta^{13}C$	-12.52	-7.77	-9.92	0.75	* ‡
USAFA (OS)	9	$\delta^{13}C$	-12.88	-9.42	-11.21	1.26	†‡
CIL	60	$\delta^{18}O$	-10.61	-5.04	-7.45	0.90	*
USAFA (CONUS)	219	$\delta^{18}O$	-12.40	-3.14	-6.85	1.60	*
USAFA (OS)	9	$\delta^{18}O$	-12.57	-4.61	-7.44	2.41	

*, †, or ‡ indicates significant difference between means

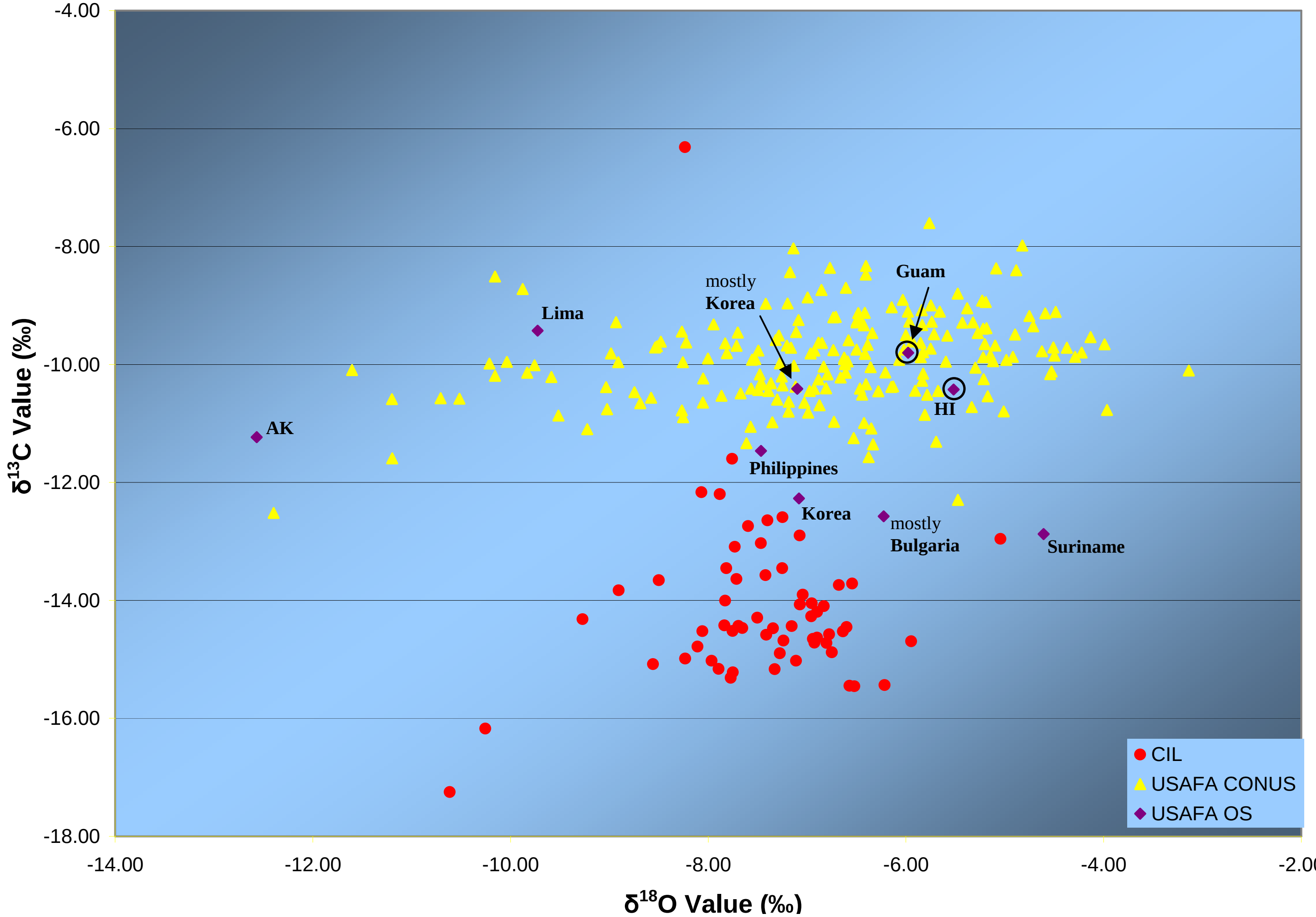

Figure 5-3. Carbon and oxygen isotope results for CONUS and overseas USAFA comparison.

the same fashion as the second run and the outcome was the same in that all group means for carbon were significantly different at $\alpha=0.05$ (Table 5-6 and Figure 5-3).

As only three individuals were added to the foreign group to create the new overseas (OS) group, (one individual each from Alaska, Guam, and Hawaii), the summary statistics did not change much between statistical runs two and three. The CIL samples represent the extreme end of the spectrum for carbon depletion, with a mean $\delta^{13}C$ value of -14.25‰. The heavy dependence of rice in the diets of humans and livestock raised for meat greatly influences the $\delta^{13}C$ values of the CIL samples, so they represent primarily a C_3 plant signature.

The modern foreign and OS individuals are all young and represent a distinct group from both the Vietnam-era CIL teeth and the American and CONUS USAFA donors. These individuals are also intermediate with a mean $\delta^{13}C$ of -11.58‰ for the foreign grouping and -11.21‰ for the OS grouping. The $\delta^{13}C$ values of -9.92‰ for both the American and CONUS participant groups are indicative of individuals with a much larger corn constituent in the diet. The influence of corn-based products have become pervasive in modern American carbon isotope ratios. Not only have Americans' consumption of whole corn increased, but corn-based snacks such as popcorn, chips, and sodas/colas sweetened with high-fructose corn syrup are essentially a staple of contemporary American diets and consumed at ever increasing rates. American consumption of meat has steadily increased over the years as well (Kantor 1998). Domestic livestock are primarily fed corn products (USDA 2006), and this base corn isotopic signature is eventually incorporated into the human tissue.

Oxygen

The CIL and USAFA samples also significantly differed with respect to their mean $\delta^{18}O$ values. The mean oxygen measure for the CIL cohort was -7.45‰ (CIL outlier excluded) while the mean for the USAFA donors was -6.88‰, with a p-value from the GLM procedure of 0.0092. No outlier from either sample group was noted. While the values between the two groups patently overlap, the CIL samples cluster much more tightly, ranging from -10.61‰ to -5.04‰ (Table 5-7). The USAFA samples completely encompass this distribution and extend out roughly 2‰ on either side, ranging from -12.57‰ to -3.14‰ (Table 5-8).

Oxygen values are dependent upon a variety of interrelated environmental factors such as latitude, temperature, altitude, distance inland, precipitation patterns, and humidity (Iacumin 1996, Hertz & Garrison 1998, Kendall & Coplen 2001). A rough latitudinal cline does appear to have emerged for the USAFA $\delta^{18}O$ values, with the higher latitudes trending more negatively and the lower latitudes demonstrating more enriched values (this will be discussed in greater detail in Chapter 6). If latitude were the dominant factor contributing to an organism's oxygen isotope signature, a similar cline should be observed for the CIL samples and areas that overlap latitudinally (Figure 5-4) should exhibit similar values.

These trends do hold true to an extent, but latitude does not appear to be the dominant factor reflected in an organism's $\delta^{18}O$ value. For instance, in looking at the East Asian geographical dispersion, one would assume individuals from Korea would have the most negative oxygen isotope ratios because the Korean peninsula is the furthest north of the natal regions sampled. The three Korean values actually fall in the middle of

Table 5-7. East Asian $\delta^{18}O$ values, in ascending order.

Identifier	$\delta^{18}O$	Location	Identifier	$\delta^{18}O$	Location
CIL-058	-10.61	Vietnam	CIL-004	-7.34	Vietnam
CIL-034	-10.25	Cambodia	CIL-003	-7.33	Vietnam
CIL-008	-9.27	Solomon Isl.	CIL-056	-7.28	Vietnam
CIL-048	-8.90	Vietnam	CIL-036	-7.25	Vietnam
CIL-040	-8.56	Solomon Isl.	CIL-026	-7.25	Korea
CIL-039	-8.50	Philippines	CIL-012	-7.24	Vietnam
CIL-029	-8.24	Korea	CIL-046	-7.16	Vietnam
CIL-011	-8.23	Vietnam	CIL-041	-7.11	Vietnam
CIL-033	-8.11	Cambodia	CIL-025	-7.07	Vietnam
CIL-017	-8.07	Vietnam	CIL-028	-7.07	Korea
CIL-022	-8.06	Vietnam	CIL-050	-7.04	Vietnam
CIL-016	-7.97	Vietnam	CIL-018	-6.96	Vietnam
CIL-015	-7.89	Laos	CIL-027	-6.95	Vietnam
CIL-052	-7.88	Vietnam	CIL-030	-6.94	Vietnam
CIL-009	-7.84	Vietnam	CIL-047	-6.92	Vietnam
CIL-035	-7.83	Vietnam	CIL-043	-6.90	Vietnam
CIL-037	-7.82	Cambodia	CIL-042	-6.90	Vietnam
CIL-049	-7.77	Vietnam	CIL-045	-6.83	Vietnam
CIL-010	-7.76	Vietnam	CIL-057	-6.80	Vietnam
CIL-013	-7.75	Laos	CIL-032	-6.78	Cambodia
CIL-023	-7.75	Vietnam	CIL-060	-6.75	Vietnam
CIL-001	-7.73	Vietnam	CIL-038	-6.68	Vietnam
CIL-007	-7.71	Vietnam	CIL-059	-6.64	Vietnam
CIL-044	-7.69	Vietnam	CIL-053	-6.60	Vietnam
CIL-014	-7.65	Vietnam	CIL-051	-6.57	Vietnam
CIL-061	-7.60	Vietnam	CIL-002	-6.54	Vietnam
CIL-024	-7.50	Vietnam	CIL-019	-6.52	Vietnam
CIL-006	-7.47	Vietnam	CIL-055	-6.22	Vietnam
CIL-021	-7.42	Laos	CIL-005	-5.95	Vietnam
CIL-054	-7.41	Vietnam	CIL-031	-5.04	Vietnam
CIL-020	-7.40	Vietnam			

the CIL range. The two individuals disinterred from the Solomon Islands, located just south of the equator, are among the most depleted, but based on latitude, one would assume they would be among the most enriched.

The oxygen values derived from bone carbonate correlate well to local meteoric water at an r^2=0.98 (Iacumin et al. 1996). When the International Atomic Energy Agency overlays for weighted annual $\delta^{18}O$ for Asia and North America (Figures 5-5 and 5-6) were consulted, it became apparent why the range for East Asia is completely

Table 5-8. Partial list of USAFA $\delta^{18}O$ values, in ascending order (30 most depleted and 30 most enriched).

30 Most Depleted Values			30 Most Enriched Values		
Identifier	$\delta^{18}O$	Location	Identifier	$\delta^{18}O$	Location
AFA-063	-12.57	AK	AFA-020	-5.19	
AFA-032	-12.40	Alberta/OR	AFA-083	-5.12	TX
AFA-226	-11.60	MT	AFA-196	-5.09	GA
AFA-136	-11.46	ID	AFA-167	-5.06	TX
AFA-121	-11.20	MT			**CIL Overlap**
AFA-212	-10.71	MT	AFA-073	-5.01	TX
CIL Overlap			AFA-014	-4.98	TX
AFA-012	-10.52	VT/ID/VT	AFA-240	-4.96	TX/CO
AFA-252	-10.30	UT	AFA-251	-4.92	AL
AFA-051	-10.21	CO	AFA-087	-4.90	GA
AFA-166	-10.18	ND	AFA-114	-4.88	TX/GA
AFA-103	-10.16	CO	AFA-205	-4.82	GA
AFA-024	-10.16	CO	AFA-139	-4.79	CO/Panama/HI/TX/Ger
AFA-099	-9.88	CO	AFA-172	-4.76	TX
AFA-145	-9.85	WY	AFA-186	-4.75	OK
AFA-211	-9.83	UT	AFA-155	-4.73	TX
AFA-194	-9.79	CO	AFA-062	-4.71	TX
AFA-146	-9.76	ID	AFA-180	-4.64	FL
AFA-109	-9.73	Peru	AFA-220	-4.61	Suriname
AFA-246	-9.70	MT	AFA-151	-4.55	TX
AFA-031	-9.52	CO	AFA-070	-4.54	FL
AFA-005	-9.22	NY	AFA-110	-4.51	TX
AFA-241	-9.13	CO	AFA-053	-4.49	FL/MN
AFA-270	-9.06	CO	AFA-128	-4.39	IL/OK
AFA-107	-9.03	OR	AFA-077	-4.37	TX/NH
AFA-026	-9.02	PA/NC/TX	AFA-272	-4.35	GA
AFA-060	-8.98	NE	AFA-003	-4.22	PR/FL/VA
AFA-079	-8.93	MT/WA	AFA-160	-4.13	FL
AFA-044	-8.91	MN	AFA-156	-4.11	TX
AFA-116	-8.84	CA	AFA-274	-3.75	FL
AFA-123	-8.75	MS/WA/AL/FL/PA	AFA-022	-3.14	CA/FL

contained within the range for the USAFA values. Even though the latitudinal gradient for the two regions is fairly disparate, the annual $\delta^{18}O$ for precipitation is quite similar for East Asia and the CONUS, with the exception perhaps of the southeast U.S. So many different factors interact to influence the $\delta^{18}O$ values for organisms that it appears local ecosystems have a much more overarching effect than generalized global features such as latitude.

One aspect that likely is confounding these results is the unknown true provenance for many of the CIL accessions or uncertainty as to the natal origins of individuals disinterred from these regions. According to the CIL's Casualty Automated Recovery and Identification System (CARIS) database, several sets of remains purported to be American servicemen were turned over to U.S. authorities by Vietnamese refugees seeking asylum in the U.S. After evaluation by CIL anthropologists, the remains were determined to be Mongoloid of foreign origin. Remains were surrendered by refugees in locations such as France, Hong Kong, Thailand, Malaysia, and Singapore, thus teeth used from these skeletons could have originated from these nations, from Vietnam itself, or elsewhere. Further complicating matters is the fact that the Pacific theater has been a hotbed of military conflict over the past century. It is quite possible, for instance, that the remains disinterred from the Solomon Islands are not of native islanders, but of fallen

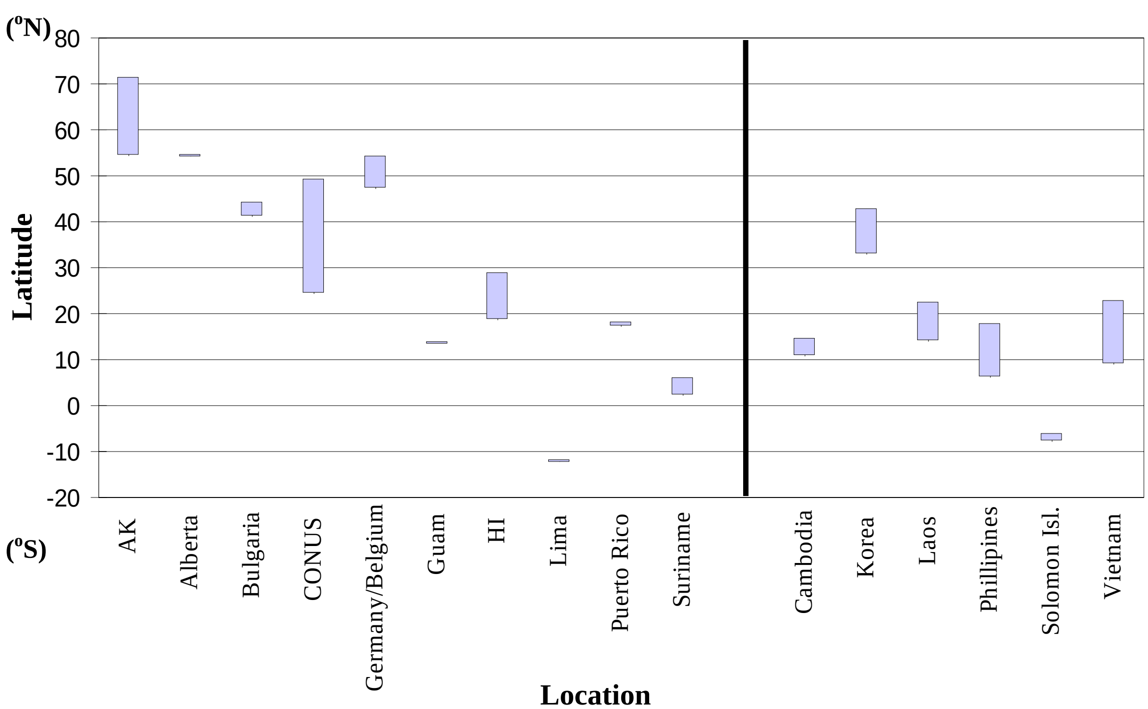

Figure 5-4. Latitudinal dispersion of major natal regions featured in this study. East Asia is on the right. Information drawn from Rand McNally Atlas (1998).

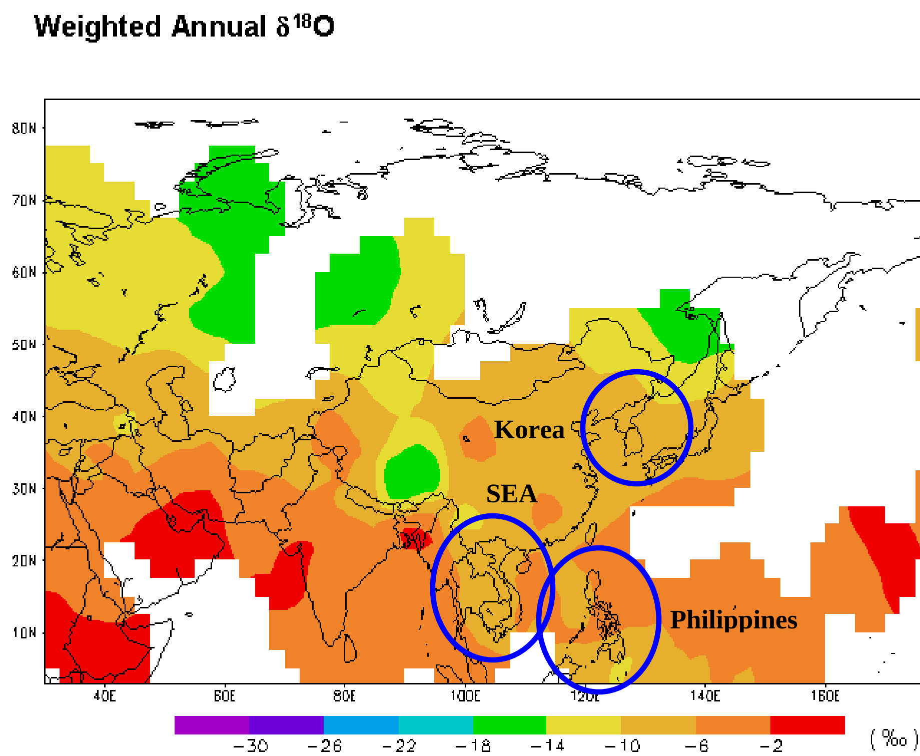

Figure 5-5. Weighted Annual $\delta^{18}O$ for Asia. Map reproduced from IAEA (2001).

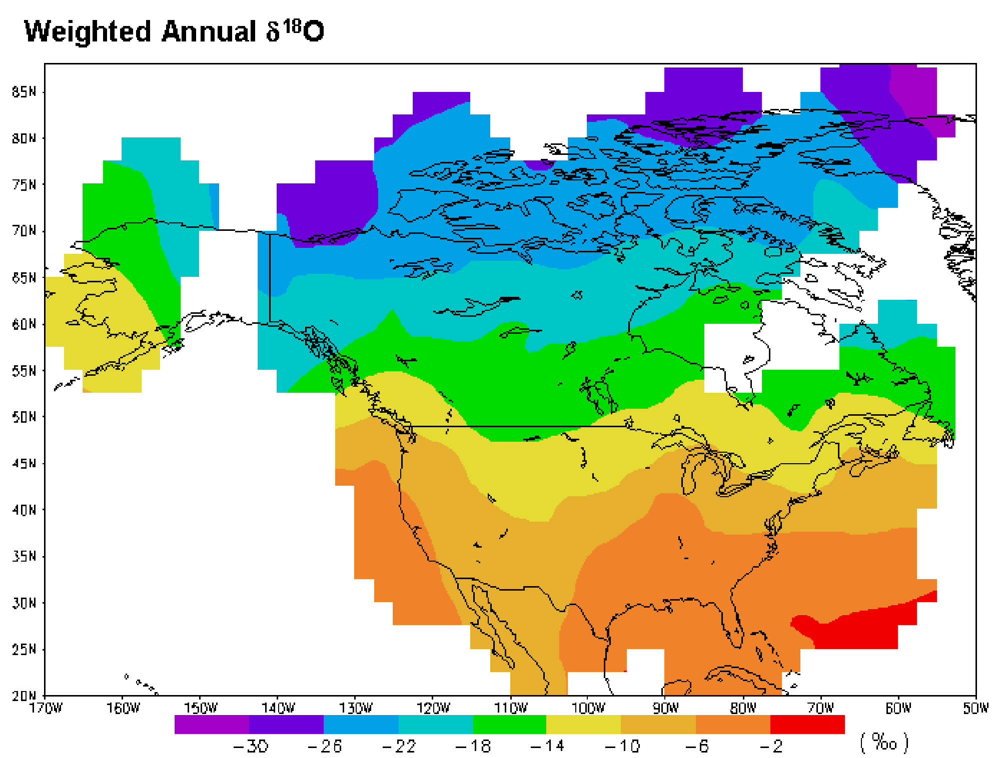

Figure 5-6. Weighted Annual $\delta^{18}O$ for North America. Map reproduced from IAEA (2001).

Philippines, in that these areas were occupied by the Japanese during World War II. The difficulties with the true origin of remains disinterred from Korea have already been addressed.

A latitudinal cline is much more apparent in the USAFA samples, although those from the European nations were much more enriched than expected, with the individual primarily from Bulgaria exhibiting a $\delta^{18}O$ value of -6.37‰ while the American raised largely in Germany and Belgium measured -5.47‰. The altitude of Lima, Peru, seems to have overshadowed its southern latitude. The $\delta^{18}O$ value of the individual from there was -9.73‰, positioning them at the lower end of the U.S. mountain states. While Guam and Hawaii were fairly enriched overall, they were not at the end of the U.S. spectrum of oxygen ratios as one would expect based on their more equatorial latitude. Their $\delta^{18}O$ values were likely influenced by island effects.

One individual each from the CIL and USAFA cohorts was purported to be from the Philippines. The CIL sample measured -8.50‰ and the USAFA sample -7.45%, more than one standard deviation apart as calculated from the East Asian pool. The enamel $\delta^{18}O$ of two USAFA individuals raised in Korea varied only by 0.02‰, measuring -7.36‰ and -7.34‰. These ratios were very similar to one CIL Korean sample with a $\delta^{18}O$ of -7.25‰. The other CIL Koreans had values of -7.07‰ and -8.24%. The individual measuring -7.07‰ is well within the standard deviation of 0.89 for the CIL oxygen values when compared to the USAFA Koreans. The -8.24‰ individual (CIL-029) is at the very cusp of the standard deviation; CIL-029 is the extreme outlier discussed in the carbon section of this chapter. The oxygen data further reinforces the notion that Korea is probably not the native origin of this individual.

Similar to the carbon series, three iterations of the GLM procedure were run to determine the influences of foreign and overseas natal regions upon the USAFA isotope ratios were run and a Tukey's studentized range test performed to control for type I experiment-wise error rate (Tables 5-1, 5-2, and 5-3). It is apparent that the removal of 6 and 9 individuals, respectively, out of a total of 228 sampled, does not notably affect the mean or standard deviation for the American group in either instance. The mean $\delta^{18}O$ for the six foreign USAFA participants was -7.15‰, a value that was intermediate to the CIL samples mean and the American USAFA samples. When the Americans reared outside of the CONUS were added to the foreign group to create the overseas assemblage, the mean $\delta^{18}O$ shifted to -7.44‰, just +0.01‰ from the East Asian mean. The standard deviation for the overseas group jumped dramatically though to 2.41‰. When the means then are compared between the three groups for the two additional iterations, only the American USAFA samples in one run and the CONUS USAFA samples in the other, are significantly different from the CIL samples at α=0.05. The foreign USAFA samples are statistically indistinguishable from the CIL and American USAFA samples as was the overseas USAFA group from the CIL and CONUS USAFA groups. For a visual representation of these three groups, see Figures 5-1, 5-2, and 5.3.

Acetic Acid Test

In addition to the main project, a side study was conducted to assess the necessity of including acetic acid in the chemical processing of samples for light isotope analysis. Acetic acid is normally used on archaeological specimens to reduce secondary carbonates within the samples resulting from interaction with the interment environment. Theoretically, acetic acid should not be required for samples extracted directly from a living subject. There is some debate as to whether the outer enamel surface does undergo

exchange with the oral environment, but there is nothing in the literature to indicate even if it does, that secondary carbonate contamination would result.

New teeth from 10 USAFA donors were drilled and the enamel powder chemically processed. Half of the powder for each tooth was processed following the standard protocol while the other half omitted the steps in which the enamel powder was bathed in acetic acid for 30 minutes and washed twice with distilled water. The differences in each set of isotope values displayed no real trend with some pairs for each isotope showing positive differences and others, negative differences (Table 5-9). Based on two-tailed, paired t-tests, both the $\delta^{13}C$ and the $\delta^{18}O$ values were found not to be significantly different between samples treated with acetic acid and those without. The carbon values had a mean difference of -0.15‰ between treated and untreated specimens, with a p-value of 0.300. The oxygen values had a mean difference of 0.19‰ between treated and untreated specimens, with a p-value of 0.311. Based on the p-values however, it should not automatically be assumed that an acetic acid wash is not necessary in treating samples from living subjects.

The machine precision for the carbon and oxygen analyses for this study averaged 0.08‰ and 0.14‰, respectively. If there truly were no difference between the treated and untreated values then the average difference should be near or below the precision value, not nearly twice the value of it, as in the case of carbon. The mean difference also exceeds the precision value for oxygen. It is difficult to say why this may be the case, since the exposure time was limited to only 30 minutes. It may be if there are no secondary carbonates to act upon, the acetic acid may instead be interacting with the

Table 5-9. Results of acetic acid test, with intertooth comparison when available.

Subject	Tooth	ID	$\delta^{13}C$	$\delta^{18}O$	Date	
AFA-015	No comparative data					
	32	ACE-001	-10.53	-10.30	10-Jan	
		ACE-002	-9.73	-9.64	10-Jan	w/o acetic
AFA-035	No comparative data					
		ACE-003	-11.33	-6.15	10-Jan	
		ACE-004	-11.10	-6.18	10-Jan	w/o acetic
AFA-043	16		-10.28	-5.83	5-Dec	
	17	ACE-005	-10.56	-6.86	10-Jan	
		ACE-006	-10.29	-6.86	10-Jan	w/o acetic
AFA-068	1		-9.51	-7.29	7-Dec	
	16	ACE-007	-9.53	-7.46	10-Jan	
		ACE-008	-9.81	-8.10	10-Jan	w/o acetic
AFA-083	17		-9.94	-5.12	10-Jan	
	32	ACE-009	-9.97	-5.64	10-Jan	
		ACE-010	-10.49	-6.23	10-Jan	w/o acetic
AFA-085	16		-9.76	-6.74	10-Jan	
	17	ACE-011	-9.68	-6.45	10-Jan	
		ACE-012	-9.56	-6.97	10-Jan	w/o acetic
AFA-088	17		-9.47	-6.34	8-Jan	
	16	ACE-013	-9.45	-6.72	10-Jan	
		ACE-014	-9.81	-7.81	10-Jan	w/o acetic
AFA-089	17		-9.66	-5.20	8-Jan	
	32	ACE-015	-9.96	-5.27	10-Jan	
		ACE-016	-9.63	-5.26	10-Jan	w/o acetic
AFA-090	32		-10.41	-6.46	8-Jan	
	16	ACE-017	-10.54	-6.67	10-Jan	
		ACE-018	-10.33	-7.02	10-Jan	w/o acetic
AFA-105	1		-10.85	-5.81	8-Jan	
	17	ACE-019	-11.58	-6.86	10-Jan	
		ACE-020	-10.89	-6.22	10-Jan	w/o acetic

hydroxyapatite lattice of the teeth, affecting the oxygen values or that there is some other explanation that is not yet understood.

Because there is a precision measure to compare against, a better way to calculate the true difference of the means is not to take the mean of the real differences of the $\delta^{13}C$ and $\delta^{18}O$ values for each treatment set, but instead, to take the mean of the absolute differences between each treatment set. Since there is no overall directionality in differences, when averaging a positive difference and a negative difference, it will drive

the mean inappropriately towards zero in this case. What we are more concerned with is the overall variation between those samples washed in acetic acid and those which were not. When this approach is taken, the mean of the absolute differences for $\delta^{13}C$ increases to 0.38‰ and for $\delta^{18}O$ jumps to 0.45‰, giving a better indication of just how disparate the acetic acid effects really are. Thus said, the results appear inconclusive at this time. This issue bears further examination, with perhaps a larger sample size shedding more light upon the situation.

Different teeth were used for the acetic acid test than for the bulk of the study. It therefore also presents an opportunity, although limited because of small sample size, to examine the effects of intertooth variation. As evidenced in Table 5-9, there were comparative values for eight individuals. Two individuals utilized for the acetic acid test were of unknown natal region and hence, were not used in the main study. All samples compared underwent the acetic acid step in processing. Like the acetic acid tests, the pairs of values showed no definite directional trends. The mean $\delta^{13}C$ difference was 0.18‰ while the mean $\delta^{18}O$ difference was calculated at 0.39‰. The results of two-tailed, paired t-tests were p-value = 0.100 for carbon and 0.047 for oxygen, indicating there were intertooth variations among third molars for oxygen at α=0.05, but not for carbon. The same argument can be made though for the carbon and oxygen values as was presented for the acetic acid test. The assessment of no statistical difference may not in fact hold true since the mean difference between teeth is greater than the precision values for the PRISM. When the means for the absolute differences of $\delta^{13}C$ and $\delta^{18}O$ are computed, they increase to 0.19‰ and 0.46‰, respectively.

It also bears mentioning that some variation of isotope ratios will arise from the bulk sampling process. Enamel is laid down sequentially in layers (Hillson 1996). When a tooth is bulk sampled, one must consider that the bulk value is itself an average figure corresponding to dietary intake and environmental conditions over the period of amelogenesis for that specific tooth. This period of crown formation can range from nearly 4 years postpartum in first molars to over 13 years postpartum in third molars in Caucasian males (Fanning & Brown 1971). This indicates that intratooth variation is something that must be considered when performing isotope analyses. This value can be fairly large, with values cited in the literature of up to 8.1‰ for the same third molar, likely arising from the bulk sampling process (Beard and Johnson 2000). It is difficult to speculate just what the degree of variation is as, "…dental tissues have not been well characterized with respect to compositional heterogeneity" (Budd et al. 2004).

Heavy Isotopes

Strontium and lead analyses of the NBS 987 strontium standard during the course of this study gave $^{87}Sr/^{86}Sr = 0.71025$ (+/-0.00004, 2 σ) (Dr. George Kamenov, personal communication). All lead isotope ratios were relative to the following values for NBS 981: $^{206}Pb/^{204}Pb = 16.937$ (+/-0.004 2σ), $^{207}Pb/^{204}Pb = 15.490$ (+/-0.003 2σ), and $^{208}Pb/^{204}Pb = 36.695$ (+/-0.009 2σ) (Kamenov et al. 2005).

Strontium

The means of the strontium ratios for the CIL (outlier excluded) and USAFA pools differed significantly, with a calculated p-value of 0.0013 (Table 5-1). The mean strontium value for the CIL teeth was 0.710995 while the mean for the USAFA teeth was 0.709273. For this isotope ratio, the CIL samples have a much broader range with values

flanking either side of the USAFA samples (Table 5-10). The USAFA samples displayed a great deal of uniformity, even though the United States varies considerably geomorphologically. The mean $^{87}Sr/^{86}Sr$ ratio for the Air Force Academy was similar to

Table 5-10. Strontium isotope values for CIL and USAFA samples, in ascending order.

Identifier	Location	$^{87}Sr/^{86}Sr$	$\varepsilon^{87}Sr$	Identifier	Location	$^{87}Sr/^{86}Sr$	$\varepsilon^{87}Sr$
CIL-032	Cambodia	0.706811	+33	AFA-109	Lima	0.707449	+42
CIL-020	Vietnam	0.708047	+50	AFA-047	Philippines	0.707483	+42
CIL-025	Vietnam	0.708568	+58	AFA-173	CA	0.707969	+49
CIL-047	Vietnam	0.708581	+58	AFA-086	HI	0.708247	+53
CIL-039	Philippines	0.708864	+62	AFA-003	Puerto Rico/FL	0.708348	+55
CIL-037	Cambodia	0.708943	+63	AFA-111	CA	0.708464	+56
CIL-033	Cambodia	0.709033	+64	AFA-184	Bulgaria	0.708569	+58
CIL-052	Vietnam	0.709052	+65	AFA-063	AK	0.708616	+58
CIL-060	Vietnam	0.709283	+68	AFA-032	Alberta/OR	0.708770	+61
CIL-001	Vietnam	0.709534	+71	AFA-004	TN	0.708783	+61
CIL-043	Vietnam	0.709577	+72	AFA-075	Ger/Belg/GA	0.708820	+61
CIL-058	Vietnam	0.709657	+73	AFA-174	KY	0.708919	+63
CIL-049	Vietnam	0.709716	+74	AFA-176	FL	0.708938	+63
CIL-034	Cambodia	0.709750	+75	AFA-163	TX	0.708971	+63
CIL-042	Vietnam	0.709872	+76	AFA-023	Guam	0.708984	+64
CIL-055	Vietnam	0.709885	+76	AFA-089	FL	0.709012	+64
CIL-054	Vietnam	0.709989	+78	AFA-025	MI	0.709149	+66
CIL-021	Laos	0.710185	+81	AFA-164	GA	0.709188	+67
CIL-005	Vietnam	0.710193	+81	AFA-134	MS	0.709264	+68
CIL-046	Vietnam	0.710207	+81	AFA-056	MN	0.709333	+69
CIL-050	Vietnam	0.710336	+83	AFA-021	AZ	0.709355	+69
CIL-004	Vietnam	0.710645	+87	AFA-116	CA	0.709388	+69
CIL-040	Solomon Isl.	0.710747	+89	AFA-133	AL	0.709439	+70
CIL-003	Vietnam	0.710781	+89	AFA-146	ID	0.709499	+71
CIL-009	Solomon Isl.	0.710798	+89	AFA-096	VA	0.709574	+72
CIL-011	Vietnam	0.710954	+92	AFA-006	CA	0.709597	+72
CIL-048	Vietnam	0.711485	+99	AFA-060	NE	0.709994	+78
CIL-012	Vietnam	0.712397	+112	AFA-085	CT	0.710012	+78
CIL-013	Laos	0.712672	+116	AFA-220	Suriname	0.710045	+79
CIL-029	Korea	0.712772	+117	AFA-017	AZ	0.710052	+79
CIL-044	Vietnam	0.713442	+127	AFA-078	VT	0.710055	+79
CIL-015	Laos	0.714369	+140	AFA-031	CO	0.710130	+80
CIL-059	Vietnam	0.714642	+144	AFA-051	CO	0.710463	+85
CIL-028	Korea	0.716412	+169	AFA-103	CO	0.710606	+87
CIL-007	Vietnam	0.718229	+195	AFA-143	Korea	0.711171	+95
CIL-026	Korea	0.721172	+237	AFA-148	Korea	0.711186	+95

the value for open ocean water (0.709165), where the isotopic concentration of Sr does not change detectably from place to place (Stille & Shields 1997). This phenomenon will be explored in greater detail in the following chapter.

Beard and Johnson (2000) state, "In order to facilitate comparison of the numerically small differences in $^{87}Sr/^{86}Sr$ ratios, Sr isotope compositions may be presented in ε^{87} notation," defined as:

$$\varepsilon^{87}Sr = ([^{87}Sr/^{86}Sr]_{MEASURED}/[^{87}Sr/^{86}Sr]_{BULK\ EARTH} - 1) * 10{,}000 \qquad (5\text{-}2)$$

where the $[^{87}Sr/^{86}Sr]_{MEASURED}$ is the measured $^{87}Sr/^{86}Sr$ and the $[^{87}Sr/^{86}Sr]_{BULK\ EARTH}$ is equal to 0.7045. The analytical uncertainty in terms of $\varepsilon^{87}Sr$ values are 0.2 to 0.4 $\varepsilon^{87}Sr$ units (Beard & Johnson 2000).

When examined this way, it is a bit easier to visualize the range of values for East Asia, from a Cambodian $\varepsilon^{87}Sr$ value of +33 to a high of +237 from an individual thought to be of Korean origin. This contrasts markedly to the USAFA samples which ranged from +42 from someone from Lima, Peru, to +95 for the two individuals who grew up in Korea. The consistency of the USAFA Korean values is interesting, although they are not quite congruent with the CIL Korean values, which ranged from $\varepsilon^{87}Sr$ = +117 to +237. It is interesting to note though, that both sets of values were at the high end for their respective sampling groups, while the values for the individuals thought to be and known to be of Filipino origin were both at the low end of the spectrum. These two individuals displayed a $\varepsilon^{87}Sr$ difference of 20 for the CIL and USAFA value from the Philippines, although compared to the other individuals sampled, they still trend fairly

closely as represented in Figures 5-7 and 5-8. Furthermore, while the two individuals disinterred from the Solomon Islands overlap with the Laotian and Vietnamese values, they cluster very tightly, both with ε^{87}Sr values of +89 ($^{87}Sr/^{86}Sr$ = 0.710798 and 0.710747).

These disparities in ε^{87}Sr values do not however, mean that the subset values of each population do not necessarily agree with each other. One must be careful when inferring information from point values for strontium isotope ratios. National or regional borders, by and large, do not conform to the delineations of geological formations. The result is that one nation or component thereof, may have several very diverse strontium values that are all equally valid. For instance, in mountainous regions there is often very young rock abutting very old rock, due to the geological upheaval that caused the

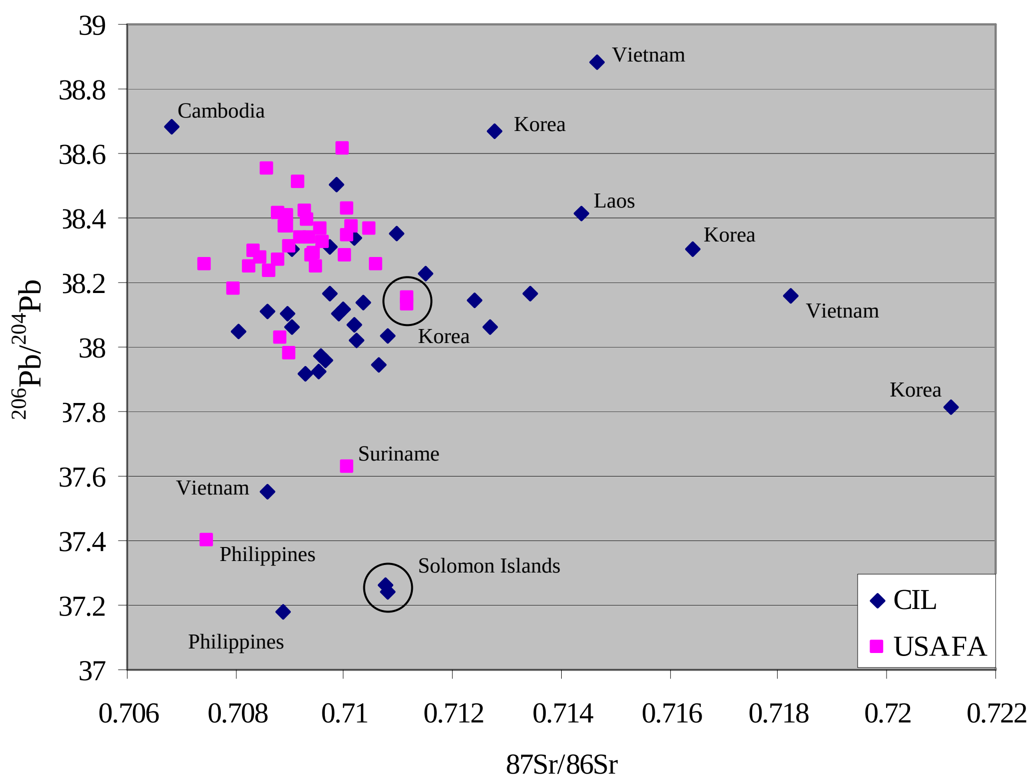

Figure 5-7. Plot of strontium values compared to $^{208}Pb/^{204}Pb$.

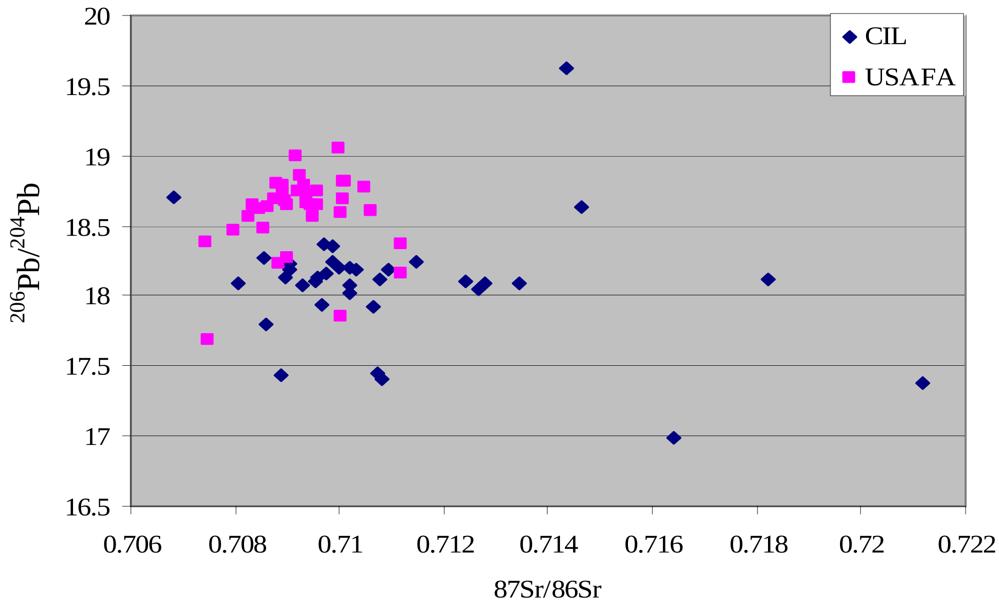

Figure 5-8. Plot of $^{87}Sr/^{86}Sr$ compared to $^{206}Pb/^{204}Pb$.

mountain formation and erosional factors. This can lead to very diverse strontium values within a very small area (Dr. Brian Beard, personal communication). This concern is echoed by Budd et al. (2004),who maintain this "…also means that simplified geological mapping and a knowledge of strontium isotope geochemistry of rocks cannot always be reliably used to estimate the expected range of food $^{87}Sr/^{86}Sr$ for any particular locality."

When examining strontium values then, it is unwise to eliminate an area simply because the strontium isotope ratios differ, unless one is certain of the geological formations underlying that specific locale and its associated $^{87}Sr/^{86}Sr$ value. If a specific area is of interest, often the best comparative method is to determine reference values for strontium via soil leachates, water sampling, or through testing the $^{87}Sr/^{86}Sr$ of fauna feeding on local resources, due to strontium's apparent lack of fractionation in biological

systems (Toots & Voorhies 1965, Ambrose 1993, Carlson 1996, Hertz & Garrison 1998, Beard & Johnson 2000, Budd et al. 2004).

Looking at the box and whisker plot (Figure 5-9), it appears that strontium does a good job of distinguishing among most of the East Asian nationalities. Cambodia and Laos are distinct groups with no overlap, even though they share a common border. The CIL Korean samples present a third discrete group if CIL-029, the outlier of questionable origin, is removed from consideration (although this is the closest value to the USAFA Korean values). While the number of individuals in each of these groups was small, the results do look promising. Twenty-three individuals believed to be of Vietnamese origin, comprised the group with the widest spread, although as previously mentioned, the true provenance in many cases is questionable.

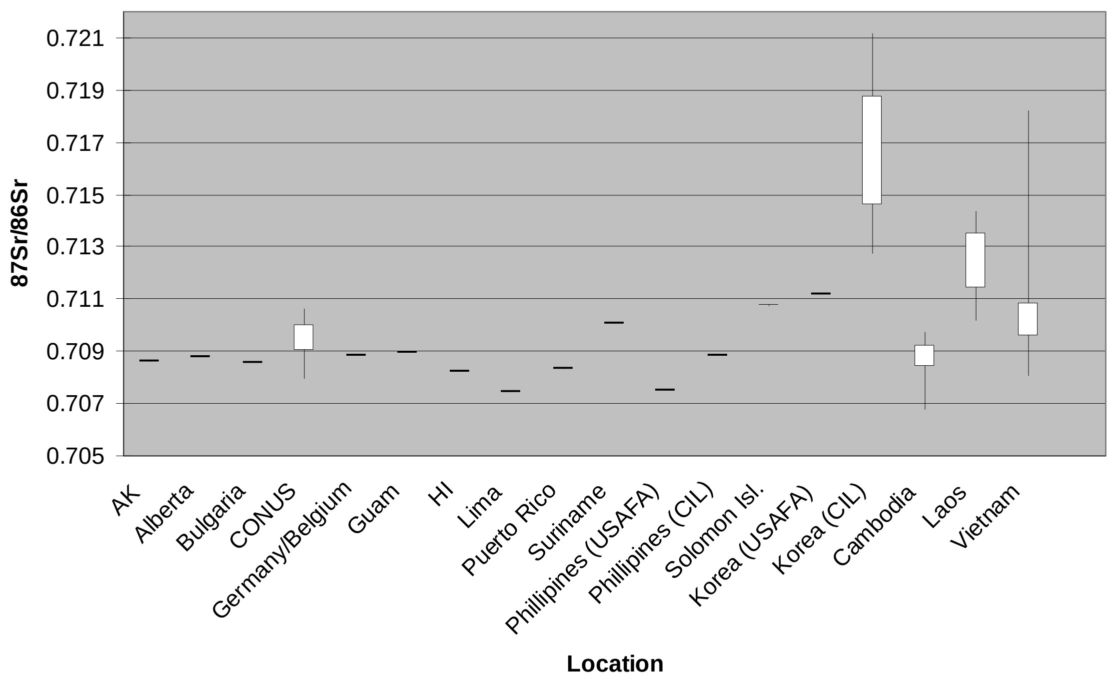

Figure 5-9. Box and whisker plot of $^{87}Sr/^{86}Sr$ values.

Table 5-11. Comparison of the means for multiple runs of the GLM procedure for $^{87}Sr/^{86}Sr$. (CIL outlier excluded.)

Population	N	LS Mean	Std Dev	
CIL	35	0.710995	0.002938	*
USAFA	36	0.709273	0.000883	*
CIL	35	0.710995	0.002938	*
USAFA (US)	30	0.709265	0.000654	*
USAFA (foreign)	6	0.709317	0.001725	
CIL	35	0.710995	0.002938	*
USAFA (CONUS)	27	0.709337	0.000642	*
USAFA (OS)	9	0.709083	0.001420	

*, †, or ‡ indicates significant difference between means

Three iterations of the GLM procedure were also run on the strontium analysis with a Tukey's studentized range test to control for Type I experiment-wise error (Table 5-11). When the USAFA cohort was split into its American and foreign constituents, it did not markedly change the mean of the USAFA group, although it did reduce the standard deviation from 0.00088 to 0.00065. A comparison of the means of these two sample sets with the East Asians revealed the only significant difference between means was among the East Asian teeth and the American teeth, at an $\alpha=0.05$. When the non-CONUS Americans were combined with the foreign individuals however, the pattern remained unchanged. Again, significant differences were only calculated between the means for the CIL and CONUS groups.

Attempts at spiking six samples to determine strontium concentrations were unsuccessful and further tests were not completed due to time and monetary constraints. Crude concentrations of the heavy isotopes however, were derived based on the solution concentration aspirated into the MC-ICP-MS, the resultant voltage generated by the solution, and the NBS-987 standard. The data is semi-quantitative at best and true values

could vary by as much as 30% to 40% (Dr. George Kamenov, personal communication) so the calculated strontium abundances hold little value themselves (see Appendix F for the raw data). They can be used comparatively however, to assess any major trends that may occur between the study populations. As can be seen from Figure 5-10, there is little difference between the strontium concentrations for the East Asians and the Academy personnel, although it does appear that the East Asians may have incorporated slightly more strontium into their tissues overall.

Lead

The MC-ICP-MS utilized for this study calculated five different lead isotope ratios ($^{208}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{206}Pb/^{204}Pb$, $^{208}Pb/^{206}Pb$, and $^{207}Pb/^{206}Pb$) and all five ratio means varied significantly between the CIL (outlier excluded) and USAFA sample groups (Table 5-1). The lead values may be found in Tables 5-12 and 5-13. The ranges of each of the lead isotope ratios for the CIL samples encompassed and extended beyond the

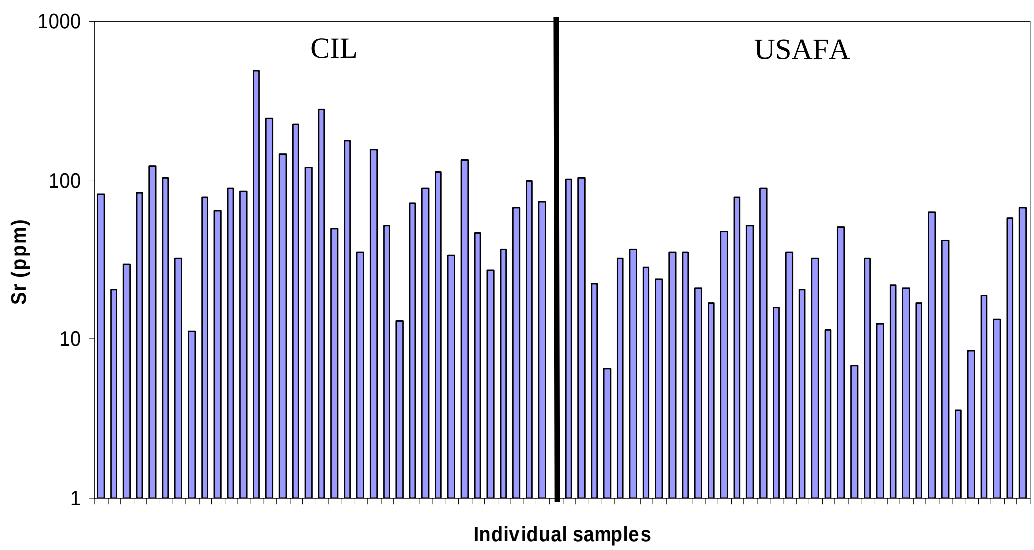

Figure 5-10. Comparative histogram of CIL and USAFA sample Sr concentrations (semi-quantitative).

Table 5-12. Lead isotope results for East Asia.

Identifier	Location	$^{208}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{206}Pb/^{204}Pb$	$^{208}Pb/^{206}Pb$	$^{207}Pb/^{206}Pb$	Pb (ppm)
CIL-001	Vietnam	37.9252	15.5873	18.0996	2.09536	0.861194	1.20
CIL-003	Vietnam	38.0377	15.6045	18.1128	2.09999	0.861511	10.95
CIL-004	Vietnam	37.9448	15.5940	17.9290	2.11644	0.869753	4.23
CIL-005	Vietnam	38.0687	15.6119	18.0142	2.11324	0.866669	6.63
CIL-007	Vietnam	38.1584	15.6223	18.1142	2.10659	0.862426	4.62
CIL-009	Solomon Isl.	37.2440	15.5288	17.4072	2.13968	0.892138	3.80
CIL-011	Vietnam	38.3545	15.6395	18.1923	2.10838	0.859685	1.06
CIL-012	Vietnam	38.1442	15.6135	18.1051	2.10681	0.862370	4.27
CIL-013	Laos	38.0637	15.6050	18.0478	2.10912	0.864676	26.11
CIL-015	Laos	38.4167	15.6958	19.6205	1.95801	0.799982	7.17
CIL-020	Vietnam	38.0458	15.5897	18.0863	2.10365	0.862003	0.38
CIL-021	Laos	38.3389	15.6322	18.1981	2.10679	0.859001	15.40
CIL-025	Vietnam	38.1107	15.5941	18.2642	2.08661	0.853807	0.77
CIL-026	Korea	37.8117	15.5544	17.3830	2.17529	0.894798	29.76
CIL-028	Korea	38.3060	15.5431	16.9917	2.25445	0.914750	154.29
CIL-029	Korea	38.6656	15.6467	18.0841	2.13804	0.865201	15.19
CIL-032	Cambodia	38.6853	15.6604	18.6979	2.06905	0.837586	42.92
CIL-033	Cambodia	38.3004	15.6251	18.2273	2.10127	0.857229	3.88
CIL-034	Cambodia	38.1621	15.6191	18.1597	2.10145	0.860097	248.03
CIL-037	Cambodia	38.1068	15.5942	18.1265	2.10229	0.860297	1.48
CIL-039	Philippines	37.1761	15.5292	17.4327	2.13262	0.890823	16.19
CIL-040	Solomon Isl.	37.2649	15.5358	17.4544	2.13498	0.890065	0.68
CIL-042	Vietnam	38.5029	15.6681	18.3566	2.09778	0.853647	1.73
CIL-043	Vietnam	37.9709	15.5875	18.1291	2.09451	0.859806	0.28
CIL-044	Vietnam	38.1647	15.6248	18.0883	2.10989	0.863783	9.48
CIL-046	Vietnam	38.0230	15.6015	18.0714	2.10403	0.863311	6.76
CIL-047	Vietnam	37.5495	15.5523	17.7935	2.11018	0.874050	3.24
CIL-048	Vietnam	38.2292	15.6148	18.2441	2.09542	0.855873	9.48
CIL-049	Vietnam	38.3071	15.6244	18.3712	2.08515	0.850476	14.40
CIL-050	Vietnam	38.1379	15.6038	18.1940	2.09623	0.857674	15.43
CIL-052	Vietnam	38.0652	15.5983	18.1806	2.09378	0.857982	4.17
CIL-054	Vietnam	38.1141	15.6007	18.1955	2.09474	0.857416	5.24
CIL-055	Vietnam	38.1058	15.5968	18.2384	2.08929	0.855162	20.07
CIL-058	Vietnam	37.9591	15.5998	17.9428	2.11556	0.869424	7.28
CIL-059	Vietnam	38.8837	15.6956	18.6266	2.08757	0.842655	4.71
CIL-060	Vietnam	37.9158	15.5826	18.0817	2.09690	0.861787	1.73

USAFA samples in both directions, but when graphed on a scatter plot, it is evident that the two populations cluster along different slopes (Figures 5-11 through 5-13). The CIL Koreans tended to separate out from the main cluster of CIL ratios and did not align with the USAFA Koreans. The values from the Solomon Islands always paired tightly and

Table 5-13. Lead isotope results for USAFA.

Identifier	Location	$^{208}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{206}Pb/^{204}Pb$	$^{208}Pb/^{206}Pb$	$^{207}Pb/^{206}Pb$	Pb (ppm)
AFA-003	Puerto Rico/FL	38.2995	15.6399	18.6511	2.05352	0.838572	0.14
AFA-004	TN	38.4127	15.6477	18.8067	2.04244	0.832003	0.06
AFA-006	CA	38.3241	15.6381	18.6474	2.05520	0.838605	0.14
AFA-017	AZ	38.4275	15.6492	18.8189	2.04192	0.831561	0.09
AFA-021	AZ	38.3369	15.6418	18.6627	2.05420	0.838178	0.11
AFA-023	Guam	37.9804	15.5983	18.2690	2.07886	0.853778	0.04
AFA-025	MI	38.5135	15.6641	18.9965	2.02739	0.824583	0.40
AFA-031	CO	38.3722	15.6484	18.8110	2.03990	0.831875	0.43
AFA-032	Alberta/OR	38.2715	15.6369	18.6870	2.04802	0.836784	0.15
AFA-047	Philippines	37.3983	15.5568	17.6810	2.11503	0.879814	0.68
AFA-051	CO	38.3673	15.6436	18.7789	2.04311	0.833015	0.14
AFA-056	MN	38.3927	15.6595	18.7845	2.04391	0.833626	0.02
AFA-060	NE	38.6165	15.6749	19.0493	2.02726	0.822855	0.08
AFA-063	AK	38.2360	15.6316	18.6329	2.05207	0.838921	0.19
AFA-075	Ger/Belg/GA	38.0297	15.6013	18.2338	2.08576	0.855654	0.14
AFA-078	VT	38.3427	15.6374	18.6884	2.05170	0.836785	0.09
AFA-085	CT	38.2804	15.6291	18.5967	2.05846	0.840443	0.17
AFA-086	HI	38.2462	15.6274	18.5617	2.06049	0.841929	0.26
AFA-089	FL	38.3107	15.6324	18.6480	2.05440	0.838312	0.24
AFA-096	VA	38.3654	15.6432	18.7485	2.04639	0.834396	0.11
AFA-103	CO	38.2570	15.6279	18.6099	2.05566	0.839792	0.32
AFA-109	Lima	38.2572	15.6141	18.3808	2.08138	0.849489	0.50
AFA-111	CA	38.2731	15.6260	18.6255	2.05501	0.838940	0.11
AFA-116	CA	38.2845	15.6308	18.7206	2.04500	0.834949	0.95
AFA-133	AL	38.2865	15.6256	18.6444	2.05352	0.838097	0.05
AFA-134	MS	38.4202	15.6564	18.8606	2.03705	0.830099	0.05
AFA-143	Korea	38.1280	15.6167	18.3654	2.07610	0.850340	0.35
AFA-146	ID	38.2489	15.6265	18.5676	2.05995	0.841478	0.06
AFA-148	Korea	38.1538	15.6119	18.1632	2.10062	0.859540	0.60
AFA-163	TX	38.4059	15.6306	18.6732	2.05679	0.837086	0.12
AFA-164	GA	38.3386	15.6357	18.7481	2.04504	0.834003	0.09
AFA-173	CA	38.1825	15.6251	18.4710	2.06718	0.845922	0.14
AFA-174	KY	38.3703	15.6463	18.7275	2.04896	0.835468	0.20
AFA-176	FL	38.3703	15.6431	18.7920	2.04169	0.832413	0.09
AFA-184	Bulgaria	38.5500	15.6315	18.4734	2.08681	0.846187	2.60
AFA-220	Suriname	37.6305	15.5767	17.8462	2.10865	0.872829	0.92

were closely positioned with the CIL ratios associated with the Philippines. One outlying value is believed to be Laotian and quite evident when the plot of $^{208}Pb/^{206}Pb$ compared to $^{207}Pb/^{206}Pb$ is examined. It is possible this person incorporated a lead signal from an object fashioned from foreign lead deposits, not encountered by others in the East Asian

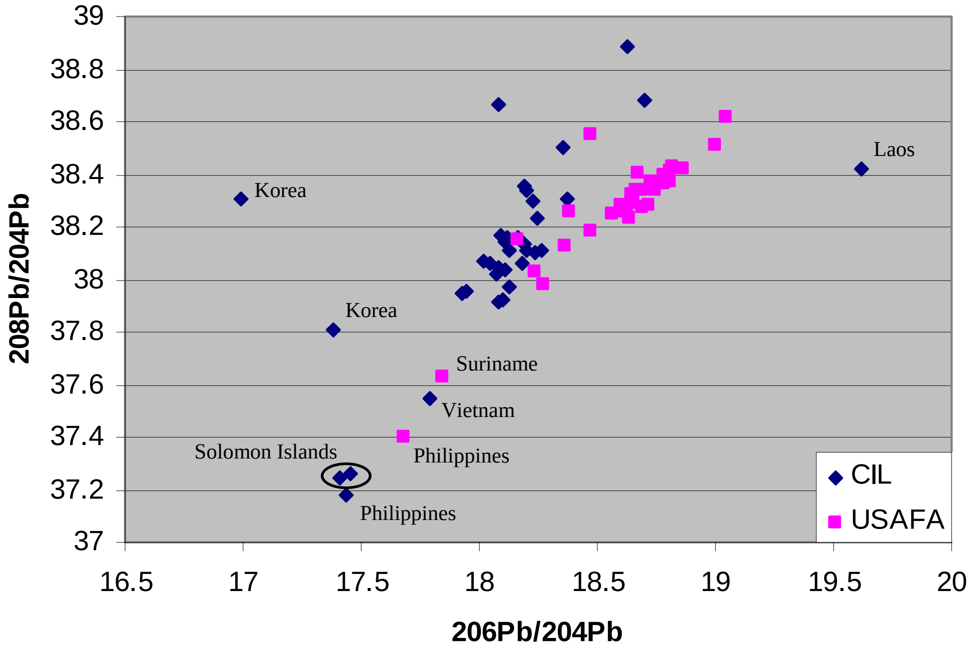

Figure 5-11. Plot of $^{206}Pb/^{204}Pb$ compared to $^{208}Pb/^{204}Pb$.

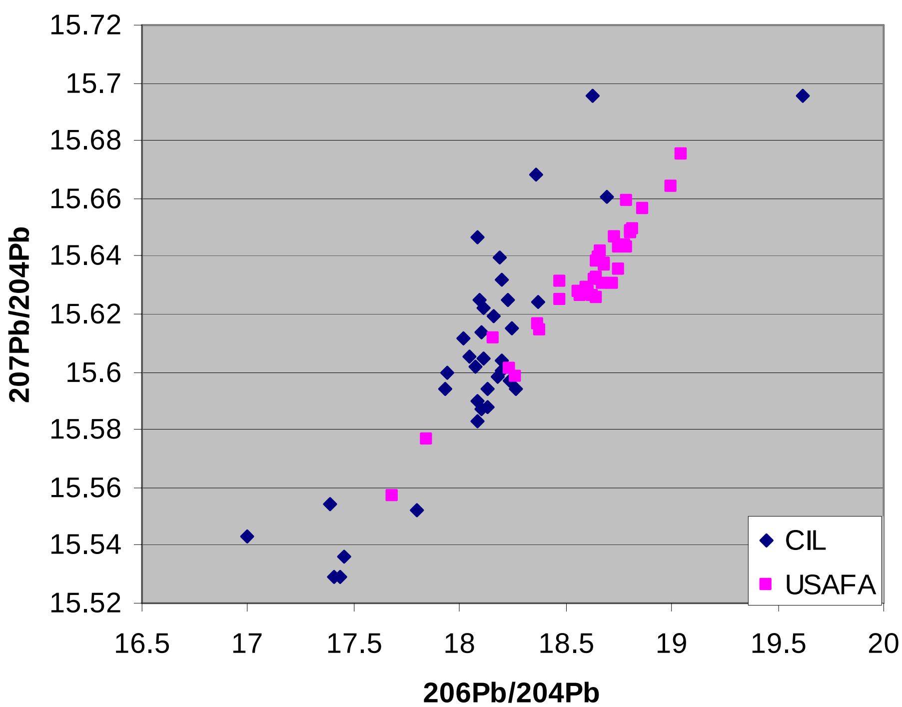

Figure 5-12. Plot of $^{206}Pb/^{204}Pb$ compared to $^{207}Pb/^{204}Pb$.

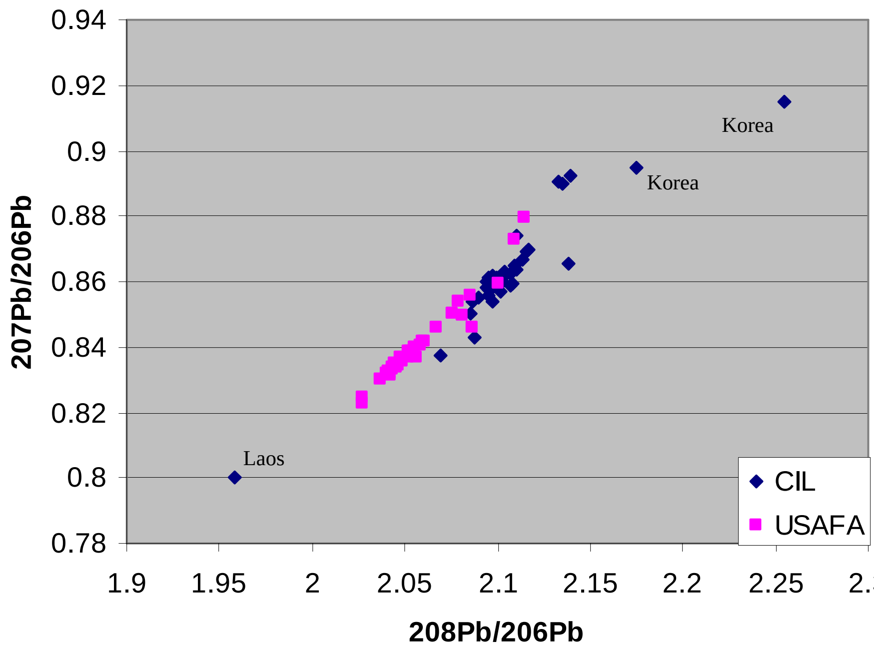

Figure 5-13. Plot of $^{208}Pb/^{206}Pb$ compared to $^{207}Pb/^{206}Pb$.

group. This individual did not have very elevated bone lead levels though, thus it is difficult to speculate further why there is such a disparity in lead values for this one sample.

Multiple runs of the GLM procedure were also performed on each of the lead ratio means in the same fashion as the other isotope ratios (Table F-3). Dividing the USAFA group into its US and foreign components produced the following results:

$^{208}Pb/^{204}Pb$	Only the East Asian and U.S. group means demonstrated significant differences.
$^{207}Pb/^{204}Pb$ $^{206}Pb/^{204}Pb$ $^{208}Pb/^{206}Pb$ $^{207}Pb/^{206}Pb$	Significant differences were found between the means of the East Asian samples and the U.S. samples and between the U.S. samples and the foreign samples. No statistical difference among the East Asian and foreign USAFA means was calculated.

Dividing the USAFA group into its CONUS and overseas components produced the same effect for all five lead isotope ratios. Significant differences were found

between the means of the East Asian samples and the CONUS samples and between the CONUS samples and the overseas samples. The East Asian and overseas means however, were statistically indistinguishable. For the table of separate group means by lead isotope, please see Appendix F.

Semi-quantitative concentrations were also calculated for lead in a similar fashion to the strontium concentrations (see Appendix F for the raw data). While the strontium showed little variation among populations, the lead content of the CIL teeth appears to be an order of magnitude or two higher than the USAFA participants (Figure 5-14). Keeping in mind the large potential for error (30%-40%), the East Asian concentrations showed a very large range of values, from 0.3 ppm to 248 ppm, with two individuals showing lead contents above 43 ppm, one at 154.3 ppm and one at 248 ppm. It is difficult to say why the lead abundances in these two individuals are so high. They have obviously had considerable environmental interaction with lead substances, perhaps through lead-based glazes on earthenware, lead pipes, or tin-soldering used in canned food items.

Lead concentrations of approximately 30 ppm in bone correspond to a blood lead level of 10 μg/dL (Smith & Flegal 1992). This blood level is considered the cutoff for lead toxicity, which often manifests itself in various developmental, skeletal, and neurological problems, especially in children (Smith & Flegal 1992). It is difficult to say how this figure directly equates to enamel values, although in a study of the lead concentration of multiple tissues, cortical bone abundances were found to be an average of 34 ppm greater than dentin for the same individuals (Bower et al. 2005).

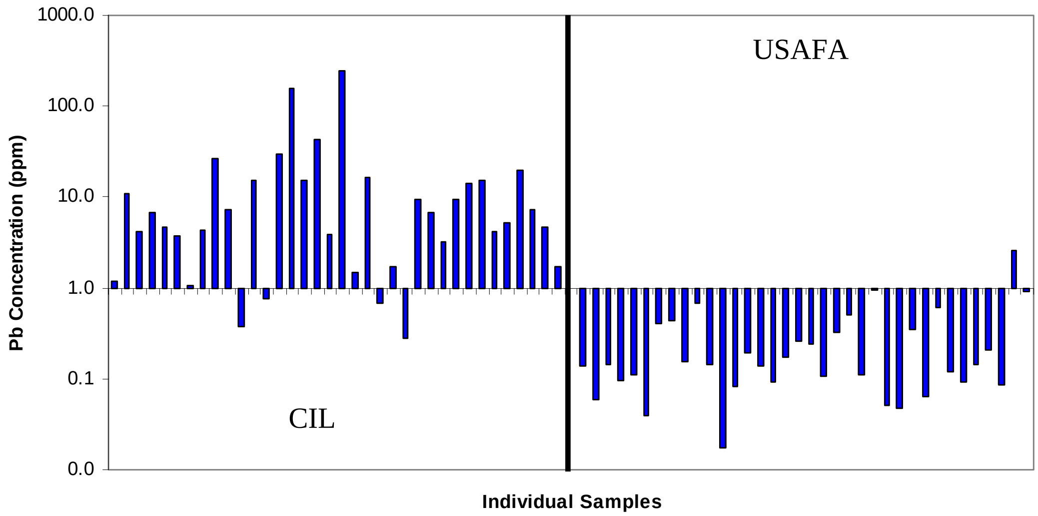

Figure 5-14 Comparative histogram of CIL and USAFA sample Pb concentrations (semi-quantitative).

Lead abundances as high as 340 ppm have been recorded in the deciduous incisors of modern children residing near a lead-smelting works in the United Kingdom (Delves et al. 1986). High lead levels were also found among 18th century Omaha Indian remains, where rib samples from 12 individuals demonstrated lead concentrations near or above 200 ppm, 3 of which were clinically unprecedented measuring higher than 800 ppm (all children), with the highest at an incredible 2,567 ppm (Reinhard & Ghazi 1992). This lead contamination may have arisen from either the practice of molding lead musket balls by mouth or through the preburial painting of corpses with red, lead-based pigments, resulting in diagenetic changes to the bone lead levels (Reinhard & Ghazi 1992).

The abundance of lead found in human skeletal remains increased between the Medieval ages and the 19th century, from low prehistoric levels to values 10–100 times higher for countries within Europe, the Americas, and Asia (Jaworowski 1990). Overall, lead concentrations in human bone decreased in the 20th century to nearly prehistoric

levels. This contrasts with rising production and environmental emissions of lead-based products though, implying that the inhalation route of lead intake makes only a minor contribution to total lead intake by humans (Jaworowski 1990).

The Air Force Academy values were, with one exception, all less than 1 ppm, with most in the range of 0.1 to 0.2 ppm. The mean lead concentration for the Academy cohort was 0.30 +0.46 ppm. In comparison, the lead content of enamel from Neolithic human teeth has been measured at approximately 0.31 $\pm$ 0.04 ppm (Budd et al. 2000). The only USAFA value above 1 ppm was 2.6 ppm and belonged to a female who primarily resided in Bulgaria and Libya. According to Dr. George Kamenov, a native Bulgarian, the town of Nova Zagora where this person grew up is a major vegetable producing area (personal communication). Assuming the inconsistency of this value with the rest of the USAFA lead contents is not a result of error on the part of the semi-quantitative estimate, it may be that the higher lead concentration in this individual is related to higher vegetable consumption. She did have the second most depleted carbon value of 222 USAFA samples run, at -12.69. It is obvious from the $\delta^{13}C$ measure that she had a very small C_4 constituent to her diet. Higher vegetable intake may well have contributed to this relatively high value although Aufderheide et al. do (1981) state, “The minute quantity of lead in plants, animals, and water does not normally exceed the capacity of human excretory mechanisms to prevent significant accumulation in the body.” Pollution effects, therefore must not be ruled out based on her residency in Eastern Europe and Libya. Regardless of why her lead concentration value is higher than the rest of the USAFA cohort, she is still within the range of normal values.

Table 5-14. Comparison of spiked lead concentration data (actual) with semi-quantitative data. (All values are in ppm.).

Identifier	Actual	Semi-quant
CIL-001	1.44	1.20
CIL-004	7.18	4.23
CIL-046	22.74	6.76
CIL-048	10.46	9.48
CIL-052	15.43	4.17
CIL-058	6.79	7.28

Six CIL samples, all thought to be Vietnamese, were successfully spiked for concentration analysis. All six values were above 1 ppm. As can be seen from Table 5-14, some of the semi-quantitative calculations were fairly close (e.g. CIL-001 or CIL-058), while others were considerably off (e.g. CIL-046). Regardless of the numerical disparity, what is evident is that the true lead concentration results demonstrate the semi-quantitative data is within the correct range of values, albeit a little low from the table data. No actual CIL values showed anything lower than 1.44 ppm. This seems to validate the finding from the semi-quantitative data, that there is a difference of at least an order of magnitude between the lead concentrations of the CIL teeth and the USAFA-provided teeth. This divergence of lead concentration values was quite unexpected and presents yet another possible avenue for distinction among East Asians and Americans.

Multi-element Approach

Because the uptake of the four isotopes discussed differs based on cultural dietary preferences, geography, and geology, it only makes sense that as you increase the number of isotopes included in your analysis, you increase the discriminatory power. An example of this is the American from Idaho who overlaps the East Asian carbon values. When the individual’s oxygen signature is examined in concert with his $\delta^{13}C$ value, it becomes clear as to which population he belongs.

One of the goals of this study was to develop a linear discriminant function for stable isotopes that would quickly allow the CIL to assess the natal origins of a set of purported American service member remains based on enamel isotope ratios. A linear discriminant function was created utilizing all of the CIL samples except the CIL outlier and the 222 USAFA participants who were raised for at least a portion of amelogenesis within the U.S. and its territories. Carbon and oxygen values were available for all of these individuals. In addition, the strontium data and measurements of all five lead isotope ratios were incorporated into the equation for 35 of the CIL samples and 30 of the USAFA samples.

The discriminant function was tested through resubstitution, whereby the same data set used to derive the discriminant function was run back through the formula (SAS 9.1). After resubstitution, 2 of 65 individuals were incorrectly classified as belonging to the wrong population. One CIL sample was incorrectly identified as being American, and one USAFA sample was misidentified as East Asian. This equated to a 97.14% correct classification rate for East Asians and a 96.67% correct classification rate for Americans, with an overall error rate for both populations of 3.08%.

The misclassified CIL sample was CIL-052, an individual recovered from Vietnam. This individual had the highest $\delta^{13}C$ value of the 35 CIL samples with values for all isotope ratios studied and the third highest value of the entire compliment of 61 East Asian samples, excluding the extreme outlier. All of the heavy isotope ratios were very near the median values for the East Asian group, although all of the lead values tracked similarly to AFA-075.

The only American misidentified was AFA-075, a self-reported Caucasian who spent the first 8 years (of 11 total) of third molar amelogenesis in Germany and Belgium. As previously mentioned, her carbon values overlapped the East Asian cluster. For every single lead value, this individual fell squarely among the foreign USAFA samples and was the minimum American USAFA value for $^{206}Pb/^{204}Pb$, the second lowest value behind Guam for $^{208}Pb/^{204}Pb$ and $^{207}Pb/^{204}Pb$, and the maximum value for $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$. Each of this individual's lead values was well within the range of all of the CIL samples.

Cross-validation was also performed to verify the discriminant function. This test is not as biased as resubstitution, since classifies each observation using a discriminant function compiled from all of the other data, so in essence, each observation is classified based on a slightly different equation. After running all of the individuals from the original data set, two of the CIL samples were misclassified as American and one USAFA sample was incorrectly classified as being of East Asian origin. This led to correct classification rates of 94.29% for the East Asian samples and 96.97% for the American samples, with an overall error rate of 4.62%. All three of the individuals already spoken to were misidentified along with CIL-015. This individual was recovered from Laos. The ^{206}Pb series for this person was significantly different than all other East Asian samples (Figures 5-11 through 5-13) with this individual's $^{206}Pb/^{204}Pb$, $^{208}Pb/^{206}Pb$, and $^{207}Pb/^{206}Pb$ values all at the end of their respective spectrum for the East Asian group. In the case of each of these three isotopes, there is a much closer affinity of this individual with the American values compared to its East Asian cohort.

This misclassification of CIL-015 and AFA-075 highlights a problem with this approach: of the eight isotope ratios used for the discriminant function, five are lead values and they are highly interrelated. Assuming equal weight of all the variables, this gives undue influence to the lead ratios over the other more discrete isotopes. To combat this, the discriminant function was recalculated using only two of the five lead isotope ratios, $^{208}Pb/^{204}Pb$ and $^{207}Pb/^{206}Pb$. These two were chosen because they represent all four stable lead isotopes. The isotopes of ^{206}Pb and ^{207}Pb reflect the signature of lead ore bodies, from which industrial lead is derived (Stille & Shields 1997). An added utility of this ratio is that it can be used as a check for anthropogenic lead contamination. These isotopes differ from ^{204}Pb and ^{208}Pb, which reflect the signature of average crustal rock, and have a markedly different lead isotopic composition from ore bodies (Stille & Shields 1997).

A new discriminant function was run utilizing only $^{208}Pb/^{204}Pb$ and $^{207}Pb/^{206}Pb$, with only slightly better results than the equation which incorporated all of the lead isotopes. The resubstitution procedure produced the exact same results as running the discriminant function with all five lead isotope ratios, with an overall correct classification rate of 96.92%. The same two individuals, CIL-052 and AFA-075 were misclassified. This formula did vary however, under cross-validation. Here, CIL-015 was correctly classified, thus the results mirrored those of the resubstitution step. To reiterate then, utilizing only two isotope ratios instead of five resulted in a 97.14% correct classification rate for East Asians and a 96.67% correct classification rate for Americans, with an overall error rate for both populations of 3.08%.

One iteration of the discriminant function was run with only the Southeast Asian data (results from Korea, the Philippines, and Solomon Islands were excluded) and two lead isotopes. This changed the numbers sampled from each population to 30 each. After a resubstitution was performed, only AFA-075 was misclassified. Correct classification rates were 100% for Southeast Asians and 96.67% for Americans, with an overall error rate of 1.67%. Cross-validation produced greater error rates as CIL-015 and AFA-075 were again misclassified, as well as CIL-052. Of the Southeast Asians sampled for all isotopes, CIL-052 recorded the most depleted $\delta^{13}C$ value (-12.20‰). All other isotope values were well within their respective ranges. With these three misclassifications, the error rates for cross-validation were 6.67% for Southeast Asians, 3.33% for Americans, and 5% overall.

While very promising, there are several points of consideration that must be addressed before this protocol is put into use. First, the sample sizes must be increased to improve the statistical robusticity of this equation. As it currently stands, this formula is based only on the isotopic results of 66 individuals total. Weights need to be calculated for each of the isotope variables to understand what the impact of each variable is upon the equation. It would also be beneficial to create a quadratic discriminant function to enable use of the formula with missing data. The USAFA data has not been proven as an accurate proxy for Vietnam-era servicemen. Additional sampling needs to be conducted from the peers of those individuals we are seeking to identify. This is essential to identify what, if any, temporal effects exist between the USAFA donors and those still listed as missing in Southeast Asia. The oldest individual sampled from the Air Force Academy was born in 1964, 3 years after the conflict in Vietnam began (Reports 1985).

Finally, a sex effect was noted for the $\delta^{13}C$ value (discussed in Chapter 6). As there are no female service members and only two civilian females listed as missing in Southeast Asia it would be beneficial to include a correction factor in the equation to account for this observation.

CHAPTER 6
VARIATION WITHIN USAFA SAMPLES

The second aim of this study was to see if regional differences in natal isotopic signatures were discernable within populations raised within the U.S. Two-hundred twenty-two individuals fit this criterion (Table 6-1). The general linear model (GLM) procedure was run on the United States Air Force Academy (USAFA) subset of American-origin to determine if there were any effects evident based on the variables generated by the survey questions. In terms of this study, origin refers to locales during the period of amelogenesis of the third molar, which ranges from age 7 to age 18 (Fanning & Brown 1971). Tests of the differences between means were run for the different responses for each question. The survey questions analyzed asked for date of birth, sex, race (open ended), tobacco use history, diet, and residency.

Year of Birth

Year of birth for the American participants ranged from 1964–1987. This age distribution lacks the older ages found in the general military population, although individuals between 18 and 25 years-old do comprise roughly 60% of the military (Friedman et al. 1989). Of the 221 individuals who provided their birth year, all but seven were born in or after 1980. Those born during the 1980s are most indicative of the ages found within the cadet wing for the academic year 2005/2006. No significant differences in birth year were found with respect to the carbon and oxygen mean values. It is highly possible though, that this is due to the large bias in the data, as 75% of all participants

Table 6-1. USAFA-provided sampling demographics, American natal region only.

Question	Response	Total	Male	Female
Participants		222	173	49
Year of birth	1964	1	0	1
	1968	1	1	0
	1973	1	1	0
	1975	1	1	0
	1976	1	1	0
	1979	2	1	1
	1980	4	2	2
	1981	5	4	1
	1982	12	8	4
	1983	45	38	7
	1984	73	57	16
	1985	47	35	12
	1986	23	18	5
	1987	5	5	0
	No response	1	1	0
Race	Native American	0	0	0
	Caucasian	177	142	35
	African American	10	8	2
	Hispanic	12	10	2
	Asian	11	7	4
	Mixed	7	3	4
	No response	5	3	2
Tobacco use	Non-user	178	133	45
	User	42	38	4
	No response	2	2	0
Diet	Meat eater	218	177	49
	Vegetarian	1	0	1
	Vegan	0	0	0
	Mixed meat/ vegetarian	1	0	1
	No response	2	1	1

were born between 1983 and 1985. Of the 30 individuals sampled for the heavy isotopes, the year groups 1964, 1975, and 1982–1987 were represented. No significant differences for the means of strontium or any of the lead stable isotope means were observed for these age cohorts.

Sex

Among the 173 males and 49 females sampled, a significant difference in mean carbon values was noted at a p-value of 0.0021. Males had a mean $\delta^{13}C$ value of -9.84‰ while females had a mean $\delta^{13}C$ of -10.21‰. No difference between the sexes was noted for oxygen. Sex differences in $\delta^{13}C$ have been noted in the literature, but these are from ancient populations where it has been hypothesized that the delta value incongruence was likely the result of different feeding regimes associated with social strata based on sex (Hedman et al. 2002). In laboratory studies, it does not appear that there is any metabolic difference between the sexes in how they assimilate carbon isotope signatures into their tissues (Schwarcz and Shoeninger 1991). For the case of the sex differences among the USAFA participants, it is likely that there is some difference in dietary intake among men and women, although all but two respondents indicated they grew up on a diet that included meat products. One female stated she was raised vegetarian, and one female indicated she switched to vegetarianism at the age of 12. The females have a more depleted mean $\delta^{13}C$, which indicates less of a prevalence of C_4-based foods in their diet. No female was found within the top 17% (most enriched) of the $\delta^{13}C$ values for the American group. This may be explained by the simple fact that while the women in this study do eat corn-fed meat, they eat proportionally less than their male counterparts, or there may be a much more complicated dynamic involved.

No sex influences were noted for strontium, but among the lead isotopes, all but $^{208}Pb/^{204}Pb$ displayed significant differences between the means at α=0.05 (the p-value for $^{208}Pb/^{204}Pb$ was 0.0523). This difference though may be more of an artifact of location, than do to an actual biological difference. Of the 30 sampled for heavy isotope analyses, 6 were female. Of these, one was the individual who spent most of her

childhood in Germany and Belgium and the other grew up in Guam. In the case of each lead isotope, these two individuals fell squarely among the foreign USAFA samples and were the two minimum American USAFA values for $^{208}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$, and the maximum American values for $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$, by quite a margin. These two individuals undoubtedly skewed the female data because of their extreme values, as the remaining four females' lead ratios were fairly well distributed throughout the group. It is doubtful then that sex played a role in the heavy isotope values.

Race

Survey respondents were asked the open-ended question as to what race they considered themselves. From the participants' responses, six race classifications emerged, Native American, Caucasian, African American, Hispanic, Asian, and mixed race. For a breakout of USAFA individuals, see Table 5-1. Of the 217 individuals who answered this question, there was no marked difference between the mean $\delta^{13}C$ values for any of the racial groups. This finding was counter to a hypothesized difference among Asian Americans, perhaps indicating less of an adherence in younger individuals to traditional Asian diets. The greatest difference between any two groups was between Asians and Caucasians, but at a p-value=0.4188 after a Tukey-Kramer adjustment, it is far from significant.

A significant difference was found for the mean $\delta^{18}O$ values between Caucasians (-6.95‰) and those of mixed ancestry (-5.14‰). After a Tukey-Kramer adjustment for multiple comparisons was completed, a p-value of 0.0316 was calculated between the two groups. The mixed ancestry group consisted of seven individuals, four of which stated they were Asian and Caucasian, two who declared they were "White/Hispanic,"

and one who answered "Korean/Mexican." All of these individuals spent at least a portion of amelogenesis in a southern state, except for AFA-162 ("Korean/White") who lived in Michigan, Maryland, and California. Within California, this individual spent 4 years in the San Francisco area. Of the other individuals, two lived in Texas, one in South Carolina, one in California and Florida, one in Texas/Michigan/Virginia, and one in Colorado/Panama/Hawaii/Texas/ Germany. The phenomenon of a much enriched $\delta^{18}O$ mean for those of mixed ancestry is probably a characteristic of this study and not indicative of the group as a whole across the United States.

For the heavy isotopes, the racial breakdown for samples run was as follows: 25 Caucasians, 4 Hispanics, and 1 Asian. No significant differences were found among the mean strontium values for the three racial categories at α=0.05. Differences were found among all of the lead isotope ratios except for $^{208}Pb/^{206}Pb$. In each instance the Asian differed from both the Caucasian and Hispanic groups. In the case of $^{208}Pb/^{204}Pb$, the differences were at p-values $\leq$0.01. No difference was noted between Caucasians and Hispanics. Because there was only one Asian sampled for lead, the differences seen are most likely locational in nature and not racially influenced. This individual was raised in Guam, where the volcanic, island geomorphology varies dramatically from the CONUS.

Tobacco Use

Two hundred twenty individuals responded to questions concerning their tobacco use history. Of these, 178 reported that they had never used tobacco products and 42 stated they had. Two individuals did not respond to this question. A significant difference among the means for $\delta^{13}C$ (-10.00‰) was noted between the tobacco users (-10.00‰) and non-users (-9.62‰), at a p-value=0.0033. No significant difference for

oxygen was observed. Tobacco interactions have not been addressed in the literature for isotopes to date.

In addition to asking whether participants had a history of tobacco use, the survey also queried those who answered yes as to what type of product was used and the frequency, in the form of an open-ended question. From this information, the following four categories were crafted for tobacco product use: no history of tobacco use; inhalant use (i.e., cigarettes and cigars); smokeless (i.e., snuff and chew); or a mixture of inhalant and smokeless products. The mean $\delta^{13}C$ value for product type was found to be significantly different between non-users and those who partook of smokeless tobacco, at a p-value of 0.0124 after Tukey-Kramer adjustment. The means equaled -10.00‰ for non-users and -9.41‰ for smokeless users. When queried as to the difference among means for $\delta^{18}O$, SAS returned a p-value of 0.0553 that at least one mean differed, but after a Tukey-Kramer adjustment, the greatest statistical difference was between smokeless users and users of both inhalants and smokeless tobacco, at a p-value of 0.0818, which does not meet the *a priori* criteria of α=0.05.

Tobacco use frequency was broken down into four categories as well: never used; used at least once a day (daily); used at least once a month but less than daily (occasional); used less than once a month (rarely). The only significant difference between means when tobacco use frequency was considered was between those who never used tobacco and those who stated they rarely used tobacco products for $\delta^{13}C$ at a p-val = 0.0042. The means were -10.00‰ for non-users and -8.87‰ for those who rarely partook of tobacco products. This does not intuitively make sense since one would think the rare-use group and never-used group would have the closest means. The simplest

explanation is this observation may actually be an artifact of small sample size for the rarely-used group (N=5) compared to the non-user group (N=178).

After examining all the data, it appears the tobacco effect upon carbon is probably due to the type of product used, versus the frequency. Sixteen individuals responded they used smokeless tobacco and an addition six stated they used both inhalant and smokeless forms. Twelve of the sixteen who used only smokeless tobacco reported they use it daily, three occasionally, and one rarely. For those who used both forms of tobacco products, four said they use them daily and two responded they rarely used them. It is possible that smokeless tobacco has an effect upon tooth isotope values because the product is in much closer proximity to the teeth for a longer period of time compared to inhalant forms of tobacco. Additionally, when the substance mixes with saliva, it is transported throughout the oral cavity and adheres more to the teeth than cigarette/cigar smoke. Tobacco products are known to stain teeth so there is obviously some interchange between enamel and the oral environment.

Concerns have been raised concerning possible diagenetic change of surface tissues in teeth (Budd et al. 1998). While no such change has been noted for strontium (Hillson 1996), Budd et al. (1998) did observe a consistent appearance of highly lead-enriched surface tissues within the first 30 μm of surface enamel. They did not believe this trait was a result of chemical exchange in the oral environment *in vivo* though, as the presence of a surface lead peak was also found in an unerupted, modern, permanent premolar, and instead attributed it to biogenic processes during tissue formation.

Heavy isotope readings did not appear to be influenced by tobacco use, since the means of the tobacco use categories did not significantly vary for any of the strontium or

lead isotope ratios. Of the 30 respondents used in this portion of the study, none reported using both inhalant and smokeless tobacco products.

Diet

All but four individuals of American-origin reported they were raised on a meat-based diet. One female indicated she was raised a vegetarian, one stated she switched from meat to vegetarianism at the age of 12 and the other two (one male, one female) did not provide a response to this question. Because of this pattern of answers, tests of differences of the means were not performed for diet. Strontium and lead analyses were not run on the vegetarian or individual who switched feeding regimes. The $\delta^{13}C$ value of the vegetarian was in the upper 40% (more enriched) of the American group, reflecting a larger C_4 constituent to the diet that the majority of the group, while the individual who switched feeding regimes was in the bottom 25% (more depleted). Both $\delta^{18}O$ values were near the mean for the American-reared cohort.

Residency

The sampled USAFA pool contained 222 individuals originating from 43 states within the United States plus the American territory of Guam. Of these 222, 43 persons lived in multiple locations within the United States, while 19 lived in the U.S. and abroad (Table 6-2). Only the states of Delaware, Kansas, Louisiana, Maine, New Hampshire, Nevada, and Rhode Island were not represented.

Strontium

Based on the successful application of strontium data to questions of region of origin in human archaeological assemblages (e.g. Åberg et al. 1998, Beard & Johnson 2000, Montgomery et al. 2005) and in a few studies of contemporary individuals (Åberg

Table 6-2. Locations during amelogenesis represented by sampled USAFA teeth.

Domestic						Foreign	
Locale	N	Locale	N	Locale	N	Locale	N
AK	1	MA	4	PA	5	Korea	1
AL	2	MD	2	SC	4	Peru	1
AR	2	MI	5	SD	1	Philippines	1
AZ	3	MN	4	TN	5	Suriname	1
CA	13	MO	1	TX	19	Int'l mix[c]	2
Locale	N	Locale	N	Locale	N	Locale	N
CO	10	MS	1	UT	2		
CT	1	MT	4	VA	3		
FL	6	NC	2	VT	2		
GA	8	ND	1	WA	2		
Guam	1	NE	3	WI	3		
HI	1	NJ	5	WV	2		
IA	2	NM	3	WY	1		
ID	2	NY	3	Unknown	1		
IL	4	OH	6	US mix[a]	43		
IN	2	OK	2	US/Int'l[b] mix	19		
KY	1	OR	5				
States Not Represented							
	DE	KS	LA	ME			
	NH	NV	RI				
[a]1 resided primarily in Puerto Rico, 2 included Guam							
[b]1 resided primarily in Belgium/Germany, 1 primarily in Alberta, Canada							
[c]1 resided primarily in Bulgaria, 1 primarily in Korea							

et al. 1998, Beard & Johnson 2000, Juarez 2005), it was hoped that strontium might prove useful for distinguishing among the Americans in this study. When the foreign-raised were removed from consideration, the uniformity of American values was even more evident (Table 6-3). While it has been estimated that the variation of ε^{87}Sr values for the CONUS ranges from +5 to +390 (Figure 6-1) due to the differences in age of basement rocks (Beard & Johnson 2000), the American samples for this study (including Alaska, Hawaii, Puerto Rico, and Guam) only extended from +49 to +80. The average value for all Americans sampled was 0.709265 (ε^{87}Sr = +68). The mean

Table 6-3. Strontium isotope values for American USAFA samples, in ascending order.

Identifier	Location	$^{87}Sr/^{86}Sr$	$\varepsilon^{87}Sr$	Identifier	Location	$^{87}Sr/^{86}Sr$	$\varepsilon^{87}Sr$
AFA-173	CA	0.707969	+49	AFA-134	MS	0.709264	+68
AFA-086	HI	0.708247	+53	AFA-056	MN	0.709333	+69
AFA-003	Puerto Rico/FL	0.708348	+55	AFA-021	AZ	0.709355	+69
AFA-111	CA	0.708464	+56	AFA-116	CA	0.709388	+69
AFA-063	AK	0.708616	+58	AFA-133	AL	0.709439	+70
AFA-032	Alberta/OR	0.708770	+61	AFA-146	ID	0.709499	+71
AFA-004	TN	0.708783	+61	AFA-096	VA	0.709574	+72
AFA-075	Ger/Belg/GA	0.708820	+61	AFA-006	CA	0.709597	+72
AFA-174	KY	0.708919	+63	AFA-060	NE	0.709994	+78
AFA-176	FL	0.708938	+63	AFA-085	CT	0.710012	+78
AFA-163	TX	0.708971	+63	AFA-017	AZ	0.710052	+79
AFA-023	Guam	0.708984	+64	AFA-078	VT	0.710055	+79
AFA-089	FL	0.709012	+64	AFA-031	CO	0.710130	+80
AFA-025	MI	0.709149	+66	AFA-051	CO	0.710463	+85
AFA-164	GA	0.709188	+67	AFA-103	CO	0.710606	+87

$^{87}Sr/^{86}Sr$ ratio is alarmingly similar to the value for sea water, at 0.709165 ($\varepsilon^{87}Sr = +66$) (Stille & Shields 1997), indicating this group as a whole is approaching the global mean for strontium or the unlikely possibility that all have diets primarily derived from the sea.

This finding seems to reinforce the original fear of the potential for erroneous conclusions due to true $\delta^{87}Sr$ values being washed out by the effects of the global economy. As stated in Chapter 1, archaeological research does not usually concern itself with such matters because food tended to be locally grown and consumed. After the Industrial Revolution and the establishment of global trade networks, food in the U.S. was very rarely grown in the localities were people lived. Younger, contemporary Americans may present a more amalgamated strontium signature because their food,

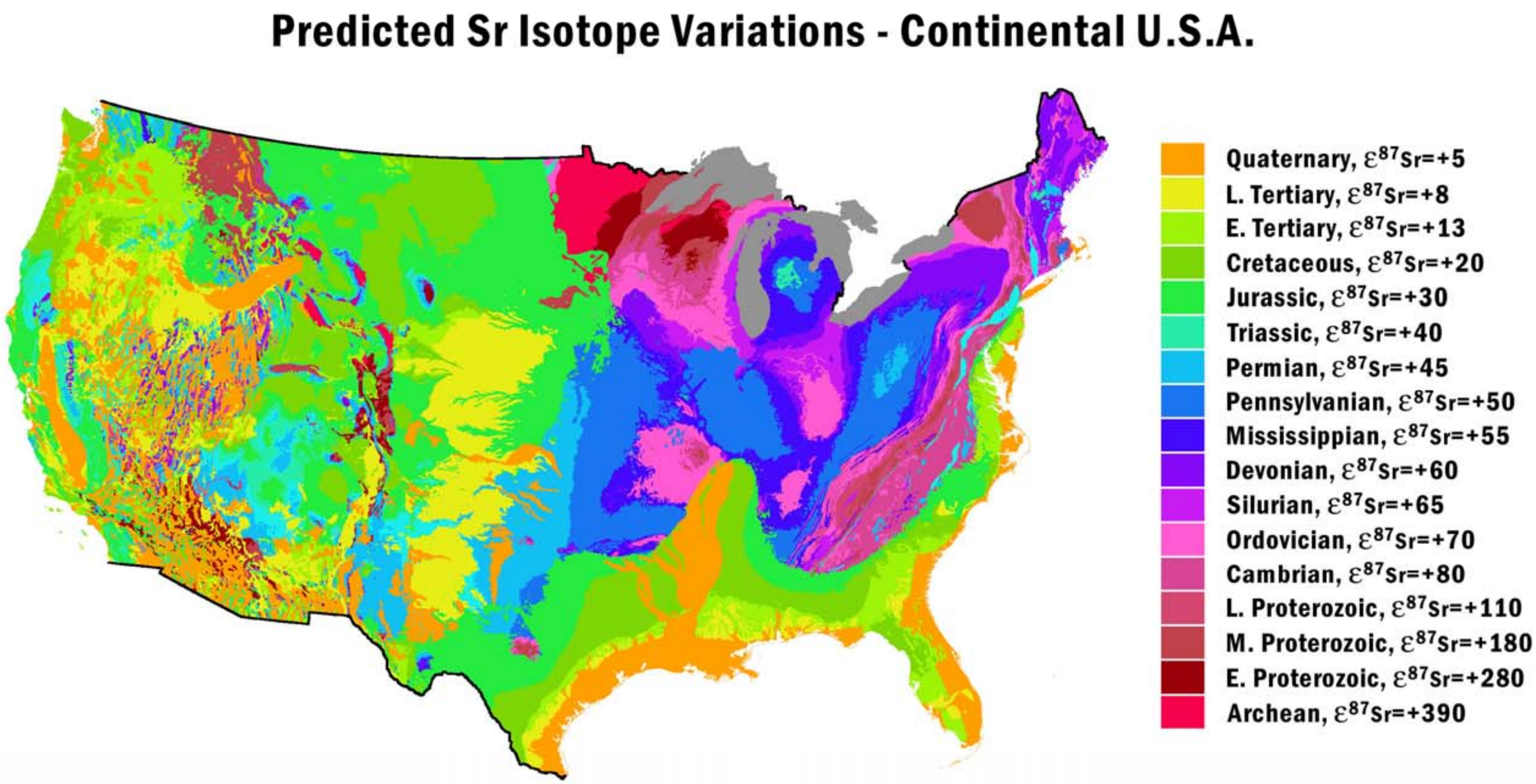

Figure 6-1. Strontium isotope composition of the U.S. showing inferred ε^{87}Sr values, as calculated by age variations in basement rocks. Image reproduced from Beard and Johnson (2000), with permission.

especially produce and some meats, comes increasingly from other states or foreign nations. Local water may provide some dietary strontium reflective of the region in which they live, but the overall effects of this world food network dietary “noise” seem quite apparent from this study and may prove difficult to overcome in strontium analyses of contemporary populations.

It would be useful to have the data from Vietnam War era individuals to see what the temporal prevalence of damped true local strontium signals are due to global dietary homogenization. It is assumed that older individuals would better reflect their natal strontium ratios, since U.S. food importation was not as wide-spread in the 1940s-1960s as compared to the subjects in this study, who largely did not begin third molar formation until the late 1980s and beyond. By further examining the variation of strontium values for the same regions during different eras, we can make a better educated opinion as to the viability of strontium analyses both spatially and temporally.

Lead

No clear trend emerged among the Americans concerning their lead isotope values. As was stated in Chapter 5, national or regional borders, by and large, do not conform to the delineations of geological formations. The result is that one state may have several very diverse values for a single lead isotope ratio that are all equally valid. Perhaps if the sample number had been increased for the heavy isotopes, a trend would have emerged or at least a better idea of why a lack of one exists. Only 30 Americans were tested, hailing from 19 states, Puerto Rico, Guam, and the two individuals who spent a significant portion of their childhood in Alberta, Canada, and the Germany/Belgium region. Of these samples, there were multiple individuals tested from four states: Arizona (2); California (4); Colorado (3); and Florida (2). In hindsight, it would have been better to

test people who had lived in the same location, but since the teeth came in over a period of 8 months, most of these relationships had not yet been established when the heavy isotope testing was being performed.

These individuals were also compared against significant lead ore deposits to rule out the lead isotope signatures being a reflection of anthropogenic contamination. Because ^{206}Pb and ^{207}Pb represent the signature found in lead ore bodies, the ratio of ^{207}Pb/^{206}Pb was used. Table 6-4 lists the ^{207}Pb/^{206}Pb ratio for nine of the major lead mining deposits and/or districts in the United States, while Table 6-5 lists the American ^{207}Pb/^{206}Pb values. As can be seen, only one of the USAFA values falls near any of the lead ore values compiled by Sangster et al. (2000). None of the individuals who lived in the same state as a major lead mining operation appear to have incorporated the mine's lead isotope ratios into their tissues.

The individual from Michigan has a similar ^{207}Pb/^{206}Pb value to the Austinville/Ivanhoe deposit in Virginia. All of the other lead isotope ratios for this person, with the exception of ^{208}Pb/^{204}Pb, are also within the respective standard errors of the lead isotope ratios for the mine. At an estimated lead concentration rate of 0.4 ppm, the person is well within the normal range, and would not have suffered from lead toxicity. It may be a coincidence or there may have been something in the home fashioned from lead from the Virginia deposit that influenced the individual's isotope signature, such as lead-based paint in an older home.

Regionality

In order to reduce the number of locales within the United States to a more manageable number, nine regions (Table 6-6 and Figure 6-2) were identified based on the δ^{18}O least squares means for location effect for the USAFA samples of American

Table 6-4. Mean $^{207}Pb/^{206}Pb$ values for major U.S. lead ore deposits.

Deposit/District	$^{207}Pb/^{206}Pb$
Upper Mississippi Valley	0.71124
Tri-State (OK/KS/MO)	0.72706
Southeast Missouri	0.74683
Central Tennessee District	0.80308
Eastern Tennessee District	0.80632
Austinville/Ivanhoe (VA)	0.82461
Red Dog (AK)	0.84710
Shafter (TX)	0.86790
Balmat (NY)	0.91929

Data compiled by Sangster et al. (2000)

Table 6-5. $^{207}Pb/^{206}Pb$ values Americans reared in the United States, in ascending order.

Location	$^{207}Pb/^{206}Pb$	Location	$^{207}Pb/^{206}Pb$
NE	0.822855	TX	0.837086
MI	0.824583	AL	0.838097
MS	0.830099	AZ	0.838178
AZ	0.831561	FL	0.838312
CO	0.831875	Puerto Rico/FL	0.838572
TN	0.832003	CA	0.838605
FL	0.832413	AK	0.838921
CO	0.833015	CA	0.838940
MN	0.833626	CO	0.839792
GA	0.834003	CT	0.840443
VA	0.834396	ID	0.841478
CA	0.834949	HI	0.841929
KY	0.835468	CA	0.845922
Alberta/OR	0.836784	Guam	0.853778
VT	0.836785	Ger/Belg/GA	0.855654

origin (N=222). After a Tukey-Kramer adjustment for multiple comparisons, the mean $\delta^{18}O$ values of location pairs were examined and those exhibiting a p-value of 1.00 were considered for consolidation. All members within a particular region share a p-value for the least squares mean for location effect of 1.00. Additionally, all states within a region are a contiguous, with the exception of region 9, which includes the islands of Hawaii and Guam. Regions are not wholly inclusive, in that some states were eligible

for membership in more than one region. For instance, Arkansas would have fit with any region from 4–9. In such instances of ambiguous group membership, states were arranged in the most latitudinally contiguous fashion. Those individuals from mixed U.S. locales were included with region 7, and those whose natal regions included international locales were included in region 8.

Table 6-6. Region membership based on $\delta^{18}O$ values.

Regions	States/Locations
(1)	AK
(2)	ID/MT/ND
(3)	CO/UT/WY
(4)	AZ/CA/NM/OR/WA
(5)	AR/OK/TX
(6)	AL/FL/GA/MS
(7)	KY/MD/NC/SC/TN/VA/WV/US mix
(8)	CT/IA/IL/IN/MA/MI/MN/MO/NE/NJ/NY/OH/PA/SD/VT/WI/US-int'l mix
(9)	Guam/HI

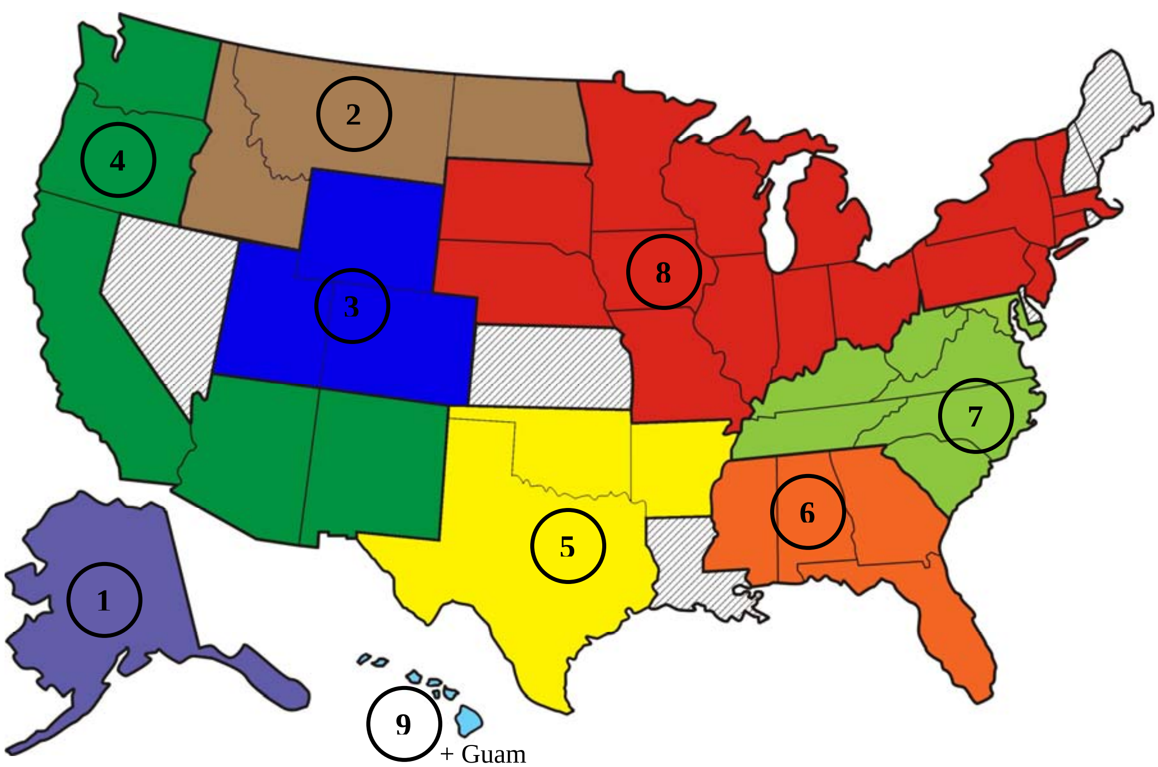

Figure 6-2. Region map based on $\delta^{18}O$ values.

When the differences between the mean $\delta^{13}C$ values for region were compared, it was discovered that region 6 (southeast) differed significantly from regions 2 and 4, with respective p-values of 0.0208 and 0.0120. Regions 3 and 4 did not differ from one another. The means for these three regions were as follows: region 2 = -10.54‰; region 4 = -10.24‰; and region 6 = -9.42‰. The results indicate individuals in the southeast have a larger C_4 component to their diets than those who reside in Idaho/Montana/North Dakota and on the west coast/southwest and may reflect regional food preferences (i.e., corn-based products such as grits [C_4] versus potatoes [C_3]). No other significant differences were found for carbon. It is interesting to note that region 8 has a mean group $\delta^{13}C$ value of -9.93‰, which is only +0.01‰ from the American mean.

As is to be expected, most of the $\delta^{18}O$ values were significantly different between regions, since the regions were created based on the $\delta^{18}O$ values for each state/location. Exceptions were expected, because as previously stated, there was some overlap between groups as well. Each region's mean $\delta^{18}O$ value overlapped with at least one other region (Table 6-7) at $\alpha=0.05$. Region 9 (Hawaii & Guam) showed the greatest homogeneity with the other regions, overlapping with five of them. Regions 7 and 8 showed mean differences at a p-value of 0.0311. All others region pairs had p-values below 0.004, 21 of which were <0.0001.

All nine regions were represented among the samples run for strontium and lead, although region membership was small since the total sample size of 30 was spread across the nine regions. After a Tukey-Kramer adjustment, two region pairs did demonstrate significant difference for their $^{87}Sr/^{86}Sr$ means. Region 3 (Colorado/Utah

Table 6-7. Region-pair comparison for difference in $\delta^{18}O$ means.

Region Pair	P-value
1 & 2	0.7105
1 & 3	0.0835
2 & 3	0.2286
4 & 9	0.1158
5 & 6	0.9908
5 & 9	0.9999
6 & 9	0.9929
7 & 9	0.9961
8 & 9	0.7855

/Wyoming) varied from region 7 (central east) with a p-value of 0.0370, and region 3 varied from the islands of Hawaii and Guam (region 9). This is heartening news, because any difference in regional values indicates that strontium signatures are not totally washed out by the global food trade. None of the region pairs exhibited significant differences among any of the stable lead isotope mean. This does not mean they do not exist. One must keep in mind that the sample size was small (three regions only had one representative) and the fact that the region break-out was based on oxygen values. It could very well be that the lead regions within the Unites States differ, thus leading to the current lead signature results. These discoveries justify continued examination of, at a minimum, strontium values to see if trends mentioned above hold true with a larger pool of samples.

An effort was made to create a lineardiscriminant function for regional affinity among the American samples. Using the 30 samples that had been run for all data and the two lead isotopes of $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$, rather than the full complement of five lead isotopes, the results proved better than anticipated. After resubstitution, the error rate for misclassification of region was 26.67%. All of the individuals from regions 1, 2, 3, 5, and 6 were correctly classified. Upon cross-validation however, the resultant

error rate increased dramatically to 66.67%. The validation process will always misclassify an individual who comprises their own group, because validation removes the observation under consideration from the pool from which the discriminant function is drawn. In the case of this study, regions 1, 2, and 5 only had one person each, and region 9 had two.

Hopefully, if the data set can be expanded so as to include approximately 10 values from each state, then regional groupings can be better drawn with more discriminating power. This analysis also highlights the fact that if the object is to maximize the effectiveness of the discriminant function, then perhaps the criteria of contiguous borders should be abandoned and Hawaii and Guam included in one of the other groups as with the U.S. mix or U.S./international groups.

Relationship Between $\delta^{18}O$ Values and Latitude

As stated elsewhere, a rough latitudinal cline appears to have emerged for the USAFA $\delta^{18}O$ values, with the higher latitudes showing more depletion and the lower latitudes more enrichment. This phenomenon has been noted elsewhere (MacFadden et al. 1999a, Kendell & Coplen 2001). In fact, the American $\delta^{18}O$ values essentially mirror Kendell and Coplen's (2001) observation that U.S. river waters are generally most depleted in Alaska, Montana, and North Dakota (less than -20‰), and most enriched in Florida and Texas (greater than 0‰). Oxygen values are fractionated in human tissue, so the river water values do not directly compare to the enamel values, but the two regions with the most depleted $\delta^{18}O$ values were region 1 (Alaska) and region 2 (Idaho/Montana/North Dakota). Additionally, the values from Florida and Texas residents were consistently the highest among the USAFA samples. The river water

values for Hawaii and Puerto Rico were high (Kendell & Coplen 2001), but not above most Florida and Texas values. The same was true of the USAFA $\delta^{18}O$ values.

To assess the effect of latitude upon the $\delta^{18}O$ values for this study, the grid coordinates for 143 individuals of American origin who resided in one location for the duration of third molar amelogenesis were examined. As can be seen, in general, as one progresses from north to south, the mean $\delta^{18}O$ values increase (Figure 6-3 and Table 6-8), except for the more equatorial islands. Variations in this latitude gradient appear to arise primarily from differences in altitude between locations found along the same line of latitude. In many instances, those latitudes with the greatest standard deviation among individuals sampled pass through areas with marked differences in altitude. This is well reflected in Table 6-8, where more often than not, the more mountainous states occupy

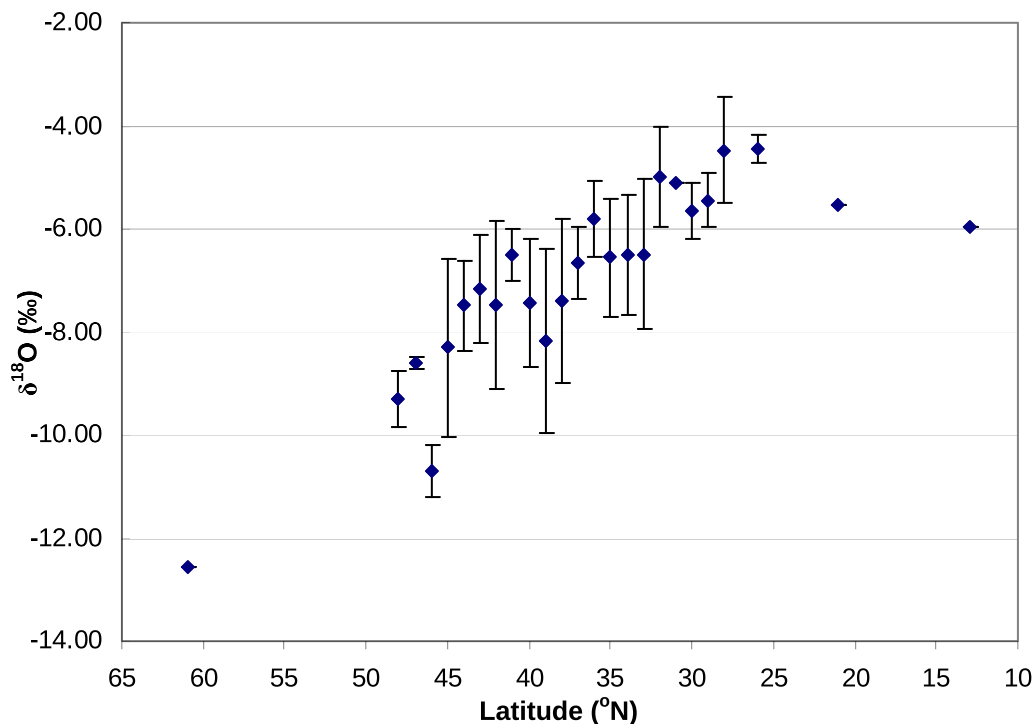

Figure 6-3. Plot of latitude compared to $\delta^{18}O$ values. Error bars equal 1 std dev.

Table 6-8. Summary statistics for American USAFA $\delta^{18}O$ values based on latitude. All values are in ‰.

				Min		Max	
Lat (°)	N	Mean	Std Dev	Value	State	Value	State
61 N	1	-12.57	---	---	---	---	---
48 N	2	-9.30	0.56	-9.70	MT	-8.91	MN
47 N	2	-8.60	0.12	-8.69	WA	-8.51	WA
46 N	3	-10.69	0.51	-11.20	MT	-10.18	ND
45 N	6	-8.31	1.72	-11.60	MT	-6.93	MN
44 N	5	-7.49	0.88	-9.03	OR	-6.87	VT
43 N	7	-7.17	1.04	-9.22	NY	-5.82	NY
42 N	11	-7.46	1.63	-11.46	ID	-6.28	IL
41 N	6	-6.51	0.50	-7.17	PA	-5.67	NJ
40 N	16	-7.43	1.25	-10.30	UT	-6.07	NJ
39 N	11	-8.17	1.80	-10.21	CO	-5.85	IN
38 N	10	-7.40	1.59	-9.79	CO	-5.55	MD
37 N	4	-6.66	0.70	-7.57	CA	-5.88	KY
36 N	5	-5.80	0.75	-6.53	TN	-4.75	OK
35 N	7	-6.56	1.14	-8.22	NM	-5.45	NC
34 N	8	-6.49	1.17	-8.84	CA	-5.41	AL
33 N	14	-6.49	1.45	-8.58	CA	-4.73	TX
32 N	6	-4.98	0.98	-6.83	NM	-4.11	TX
31 N	1	-5.12	---	---	---	---	TX
30 N	4	-5.65	0.54	-6.15	TX	-4.98	TX
29 N	7	-5.45	0.53	-6.41	TX	-4.76	TX
28 N	2	-4.48	1.02	-5.20	FL	-3.75	FL
26 N	3	-4.44	0.27	-4.64	FL	-4.13	FL
21 N	1	-5.52	---	---	---	---	HI
13 N	1	-5.98	---	---	---	---	Guam

Latitudinal information drawn from Rand McNally Atlas (1998) and Google Earth (2006)
Table fashioned after MacFadden et al. (1999a)

minimum $\delta^{18}O$ positions for a specific latitude, while lower elevations make up the maximum values.

A correlation function was performed to examine the relationship between latitude and the $\delta^{18}O$ of human enamel. Based on 142 values input into this procedure (Guam was omitted because of an eastern longitude), latitude was shown to have a weak negative correlation with $\delta^{18}O$, at $r^2 = -0.41$. This data was in line with Kendall and Coplen (2001) who found a strong negative correlation ($r^2 = -0.79$) for the eastern U.S.

and a weak relation in the west (r^2 = -0.35). This information begs the question how do altitude and annual precipitation correlate to $\delta^{18}O$ values in comparison to latitude.

Longitude for the same data set was found to be negatively correlated as well, at r^2 = -0.2642. Longitudinal impacts upon human isotopes, are not frequently mentioned in the literature. One exception is Millard et al. (2004), who declared of oxygen isotopes, “Considering the scale of the variation, it is evident that it is potentially possible to pinpoint a person's place of origin with a useful resolution, particularly with respect to east-west differences.” The most reasonable explanation within the United States is that the major mountain ranges run north to south, with the higher elevation Rockies to the west of the older, more weathered Appalachians. It has already been established that altitude has a definite impact upon oxygen isotope ratios, and the fact that the higher altitudes are in the west will affect the appearance of longitudinal influence upon group $\delta^{18}O$ values. This longitudinally-influenced $\delta^{18}O$ trend has also been observed for Great Britain, although the values tend to generally decrease as one goes from east to west (Budd et al. 2004), whereas the cline is reversed in the USAFA American values, which generally increased from east to west.

Duplicate Residences

Within this study, there were nine cities in which more than one individual resided during the entire period of amelogenesis. This presented a unique opportunity to examine how closely the oxygen values matched (Table 6-9 and Figure 6-4). It is assumed that people from the same city should have similar oxygen values since they are primarily influenced by climate and geography. If the machine precision of the PRISM for $\delta^{18}O$ was 0.14‰, then any difference between values from the same locality greater than

0.14‰ shows a variation in values that cannot be attributable to the mass spectrometer. The difference beyond this value is assumed to be attributable to some underlying

Table 6-9. $\delta^{18}O$ values corresponding to cities in which multiple participants resided. (All values in ‰.)

Identifier	$\delta^{18}O$	City	State	Std Dev
AFA-017	-7.83	Phoenix	AZ	
AFA-225	-7.14	Phoenix	AZ	0.49
AFA-116	-8.84	Los Angeles	CA	
AFA-250	-6.73	Los Angeles	CA	1.49
AFA-223	-8.58	Laguna Niguel	CA	
AFA-006	-8.27	Laguna Niguel	CA	0.22
AFA-029	-5.75	Houston	TX	
AFA-181	-5.44	Houston	TX	
AFA-163	-5.36	Houston	TX	
AFA-172	-4.76	Houston	TX	0.41
AFA-031	-9.52	Denver	CO	
AFA-065	-6.79	Denver	CO	1.93
AFA-194	-9.79	Colorado Springs	CO	
AFA-241	-9.13	Colorado Springs	CO	
AFA-270	-9.06	Colorado Springs	CO	
AFA-265	-8.25	Colorado Springs	CO	0.63
AFA-093	-7.13	Cincinnati	OH	
AFA-235	-6.28	Cincinnati	OH	0.61
AFA-051	-10.21	Arvada	CO	
AFA-024	-10.16	Arvada	CO	0.04
AFA-112	-8.22	Albuquerque	NM	
AFA-013	-8.00	Albuquerque	NM	0.16

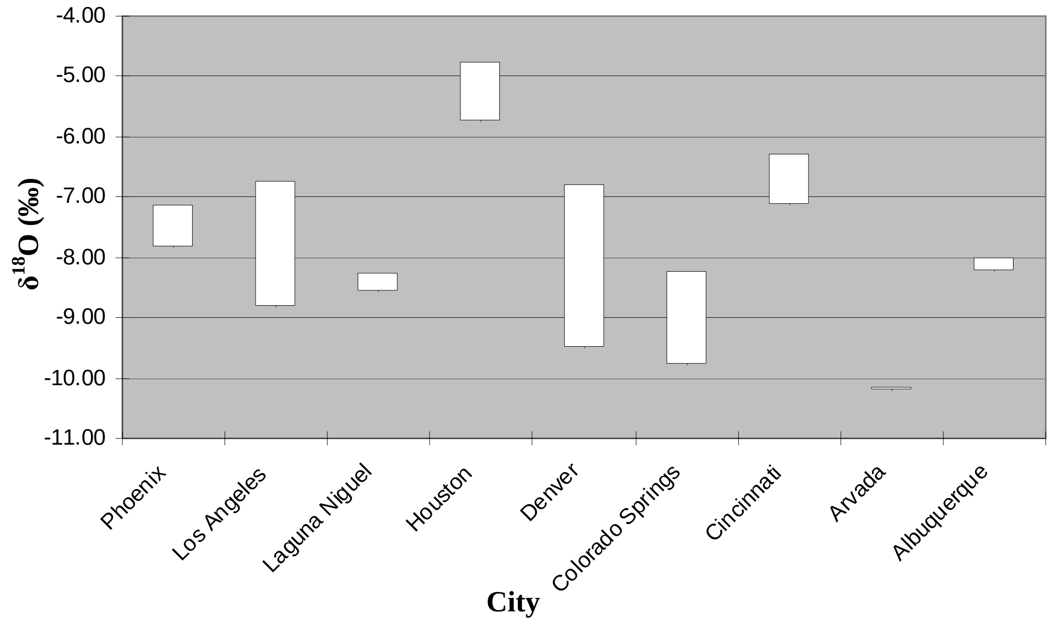

Figure 6-4. Range of $\delta^{18}O$ values produced from this study for specific cities.

biological or cultural factor independent of residence. The likelihood of differences arising due to physiological reasons is minimized because only third molars were used, ages of individuals were similar, and all were presumed to be of good health, a requirement of military service. The two residents from Arvada, CO, had surprisingly close values with difference in $\delta^{18}O$ of only 0.05‰. The two individuals from Denver, CO, on the other hand had the largest difference of 2.73‰. The Denver value of -6.79‰ seems a bit enriched for someone belonging to a mountain state and could be attributable to differential oxygen fractionation compared to the general populace, however unlikely, or the individual may have been reliant on bottled water that was not from a local source.

Comparison to the Literature

While sparse, some studies of contemporary individuals or fairly recent historic remains are available to which a comparison of the USAFA data can be made. One such study was Beard and Johnson's (2000) attempt to identify the remains of three commingled individuals from the Vietnam War. Background information on the three

indicated one was from north-central California, one Detroit, and the other spent their childhood in Vermont and Massachusetts. The individual they identified from north-central California matched surprisingly well with the USAFA donor from Mt. Shasta, California (also in the north-central portion of the state). The strontium compositions calculated by Beard and Johnson (2000) based on bedrock geology led the researchers to conclude that these individuals should have ε^{87}= +30 to +60 if they were from this locale. The Vietnam veteran has a mean third molar value (one tooth, three samples) of 0.70850 (ε^{87}=+57) while the USAFA cadet measured 0.70846 (ε^{87}=+56). This could indicate one of several things: either the effects of strontium homogenization from global food importation into the United States is not as prevalent as originally thought; the effects were being felt as early as the 1950s-1960s, when Vietnam veterans were undergoing third molar amelogenesis; or this specific person ate a high percentage of locally grown food.

Beard and Johnson (2000) where unable to distinguish among the other two individuals in their collection of remains. One presented strontium values of 0.71180 (ε^{87}=+104 for a third molar and a mean of 0.71153 (ε^{87}=+100) for another. A third molar from the last individual had a mean of 0.71062 (ε^{87}=+87). One of these persons was from Detroit, Michigan. In the present study there was participant from Rochester, Michigan, some 27 miles from Detroit (Google Earth 2005). Unfortunately, the strontium value for the USAFA molar measured 0.70915 (ε^{87}=+66), far from either of the veteran values, and outside the calculated strontium isotope composition range of ε^{87}= +90 to +200 for that area. Moreover, the USAFA value is even further off when compared to deer antler from Michigan analyzed by Beard and Johnson (2000), mean value 0.71373 (ε^{87}=+131). There

also was a USAFA donor from Vermont who measured 0.71006 (ε^{87}=+79). This is closest to the unknown veteran with a mean value of 0.71062 (ε^{87}=+87). One unaccounted for individual resided in both Vermont and Massachusetts, but it is difficult to say what the influence of the second locale would be on the overall strontium ratio, thus it is inappropriate at present to conclude that the mean value represents someone from Vermont. Strontium analyses were not run on any USAFA samples from those whose natal state was Massachusetts.

Carlson (1996) performed an examination of the lead isotope ratios of eight unidentified skeletons from a 19th century fur trade cemetery in Alberta, Canada. One of the individuals analyzed was a male Caucasian believed to have been between the ages of 23–25 when he died. USAFA-032 is a male Caucasian who lived in Alberta until the age of 14 and was 22 at the time his third molars were extracted. While the bone of the fur trader was sampled versus a tooth for the Academy personnel, because lead does not fractionate, the values should be comparable. Based on the values presented in Table 6-10, it can be seen that only the ^{207}Pb/^{204}Pb lead ratio overlaps in these two individuals. All the other lead values show quite a level of divergence. Of course, the bone lead signature of the fur trader represents a more recent biogeochemical signal than the enamel of the USAFA sample, because of its turnover rate. There is no documentation to prove the fur trader was a native to the area or even permanently resided in Alberta at the time of his death. The USAFA individual did move to Oregon at the age of 14, and hence his isotope values could represent and amalgam of the two residences. Additionally, the older sample is much more susceptible to diagenetic contamination because of the porosity of bone contributing to exchange with the interment environment.

Table 6-10 Comparison of Alberta fur trader lead values to USAFA donor from Alberta

Time Period	Sample	$^{208}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{206}Pb/^{204}Pb$	$^{208}Pb/^{206}Pb$	$^{207}Pb/^{206}Pb$	
2005	AFA-032	38.2715	15.6369	18.6870	2.04802	0.836784	
1835-1856	Fur Trader	38.45	15.62	18.44	2.085	0.847	*

*Carlson (1996)

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CHAPTER 7
SUMMARY/CONCLUSION

This study was initiated with the hope of assisting forensic investigators in identifying remains of unknown or uncertain provenance, with the explicit goal of contributing to the Joint POW/MIA Accounting Command-Central Identification Laboratory's mission of identifying those still unaccounted for from previous conflicts, and specifically, the Vietnam War. The goal was not only to distinguish isotopically between Southeast Asian and American dental remains, but to pinpoint, at least to a regional level, where in the United States an individual was reared.

Unfortunately, high impact crashes and the destructive forces of modern weaponry frequently reduce what was once an intact body to a handful of remains, rendering most conventional identification methods inadequate. Matters may be further complicated by the advance of time, unknown whereabouts of a body, and/or commingling of multiple military, terrorist, and/or civilian remains. While DNA mapping technologies are now routinely employed in identification operations, unless there are antemortem or familial profiles to compare them against, such procedures are of limited utility. Furthermore, remains are often too fragmentary or damaged to perform DNA extraction.

Nowhere is this better exemplified than by the difficulties encountered in the identification efforts for those who perished during the 11 September 2001 terrorist attack on the World Trade Center. While nearly 20,000 individual samples were recovered from the area in and around the Twin Towers, the physical destruction was so great, that only 1,592 individuals out of a presumed 2,749 who died have been positively identified

(Messer 2006). Likewise, similar difficulties are encountered by anthropologists attempting to match a name to the remains of those service personnel from previous conflicts who still remain missing.

For this project, the stable isotopes of carbon, oxygen, strontium, and lead were examined. Combined, they account for cultural dietary practices, climate and geography of natal areas, and the underlying geology of where an individual was reared. Enamel from the teeth of 61 individuals believed to be of East Asian origin, spanning the time period from World War II to the Vietnam War was collected from the Joint POW/MIA Accounting Command-Central Identification Laboratory's (JPAC-CIL) "Mongoloid" hold collection. The associated isotope values of these samples were compared to those of the enamel from the extracted third molars of 228 recent patients of the Unites States Air Force Academy (USAFA), Department of Oral and Maxillofacial Surgery. Since the USAFA participants were all living subjects, they were able to complete surveys detailing physiological, behavioral, and residential information that affect isotope values.

The least squares means for all isotope values examined exhibited significant differences between the CIL and USAFA cohorts based on a conservative multivariate analysis of variance. This appears to demonstrate that all isotopes considered are potentially useful in distinguishing between these two populations. The carbon isotopes, reflecting dietary practices, were the most discriminatory of the four examined. The Air Force Academy values were more enriched indicating a heavier C_4 component to the diet, likely due to considerable corn consumption. The East Asian values were more indicative of a C_3 diet, undoubtedly related to their dietary reliance upon rice. When the extreme CIL outlier was excluded from comparison, only six USAFA values overlapped

with the main cluster of USAFA values. Of these overlapping values, all but one corresponded to individuals reared primarily outside of the United States.

The East Asian oxygen values clustered relatively tightly, while the American values had a much greater range, extending approximately 2‰ on either side of the East Asian samples. The wide latitudinal breadth of the United States is likely the primary influence for this effect. For all the strontium and lead isotopes, the range of East Asian values encompassed the American values.

A linear discriminant function was created using all eight isotope ratios ($\delta^{13}C$, $\delta^{18}O$, $^{87}Sr/^{86}Sr$, $^{208}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{206}Pb/^{204}Pb$, $^{208}Pb/^{206}Pb$, and $^{207}Pb/^{206}Pb$), that correctly classified individuals, through resubstitution and cross-validation, as belonging to one of these two groups by 95% or better. A second version of the discriminant function was computed utilizing only $^{208}Pb/^{204}Pb$ and $^{207}Pb/^{206}Pb$ for the lead component of the equation instead of all five lead isotopes. This changed improved the overall accuracy rate of correct classification of an individual as East Asian or American to nearly 97% after both resubstitution and cross-validation. A third discriminant function was run attempting to distinguish individuals from Southeast Asia from the United States. While the same individuals were misclassified for the second and third discriminant functions calculated, because of smaller sample size in this instance, the third iteration produced a correct classification rate of 95%.

Among those USAFA donors who resided for at least a portion of the period of amelogenesis of the third molar within the United States (henceforth referred to as Americans), significant differences were found for the mean values of several of the survey variables. The sexes differed as to their carbon ratios with females displaying

more enriched values than males, likely due to differing diet preferences and not biological factors. Significant differences were also noted for $\delta^{13}C$ means among those who have never used tobacco products and those who partook of smokeless tobacco. It is possible that because this product mixes with saliva and is in close proximity to the teeth within the oral cavity that some form of chemical exchange with the enamel is occurring *in vivo*. This is the first time this phenomenon has been noted however, thus there is much speculation as to the mechanisms behind the observation.

As was feared the American strontium values displayed a distinct trend toward homogenization, with the mean value for $^{87}Sr/^{86}Sr$ varying only slightly from that of seawater. This indicates that the widespread importation of foodstuffs into the United States has had a dramatic effect upon population strontium ratios. While this may dampen the utility of this isotope for geolocational purposes within the United States, it still may prove useful when examining geographically diverse populations and may serve as a temporal indicator when populations from varied periods are compared.

In order to identify natal origin among Americans, nine regions were created within the United States based on $\delta^{18}O$ values. Good discrimination was noted between the mountain states and the southern states. A discriminant function analysis proved disappointing though, and at this point, additional sampling from most states is needed to improve the statistical robusticity of the model. Interrelated to this, a definite latitudinal cline was observed, with $\delta^{18}O$ values becoming more depleted as one moves north.

If forced to choose between the light and heavy isotopes, a combined carbon and oxygen analysis provides the “biggest bang for the buck,” both in terms of money and time, when compared to strontium and lead analysis. One pass through the mass

spectrometer provides information pertaining to cultural dietary practices ($\delta^{13}C$) and geographic location ($\delta^{18}O$). Materials required are less numerous and less costly and from start to finish, chemical processing can be done a few days sooner, with much less manpower. When at all possible, however; it is highly recommended that an investigator employ as many stable isotopic elements as possible.

One cannot use a single traditional geolocational isotope such as oxygen, strontium, or lead singly to prove conclusively than an individual is from a given area. In making attributions it can only be said that the person in question grew up in one particular area or from some other regions which are geochemically similar (Brill & Wampler 1965). If the comparative sample is sound though, a lone isotope value does permit one to make a conclusive negative judgment. With a multi-element approach however, the odds are dramatically increased that region of origin can be pinpointed, especially within a closed population.

The two greatest weaknesses of this study are the lack of American Vietnam-era samples and the requirement for greater numbers of samples from each state/territory within the United States. While the data generated from the USAFA samples is overwhelmingly positive, this sample set has yet to be proved as an appropriate proxy for Vietnam-era servicemen who still remain unaccounted for. Food consumption patterns have changed measurably within the United States over the past 40 years (Kantor 1998). Bottled water and prepared beverages are now standard fare. A global economy guarantees the exchange of foodstuffs world-wide and lead-based products are still found in high prevalence (Sangster 2000). The utility of stable isotope analyses is predicated upon the widely held assumption that you are what you eat. Thus the isotopic ratios

calculated through mass spectrometry must bear direct relationship to the food and drink ingested and imbibed by the individual over the course of their lifetime. We, as members of the human species though, especially in today's global climate, do not necessarily eat from which we live.

The present study has barely scratched the surface of the possible avenues to explore with stable isotope analyses in the medico-legal realm. Some future areas it would be interesting to explore include how the lead isotope signatures and content in enamel have changed over the course of the last 100 years in this country, especially after lead-based gasoline and paints were phased out. How do dental restorations affect stable isotope readings? Does soaking teeth in jet fuel or burning them, as might happen in an aircraft crash, impact dental isotopic signatures? How do altitude and annual precipitation correlate to $\delta^{18}O$ values in comparison to latitude and longitude? One thing is certain; individuals of a much greater range of ages must be included in isotope studies if stable isotopes are going to be used for general geolocational purposes. Hopefully, with the continued assistance of the Veterans Affairs Dental Clinic, an adequate sample set can be acquired.

This study is novel in that it is the first of its kind to compile a reference sample of isotopic values associated with known natal regions to be utilized in forensic work. More importantly, the information gleaned from this study will be applied in support of the JPAC's mission to achieve the fullest possible accounting of all Americans missing as a result of our nation's past conflicts. The databases compiled here and the analyses performed will boost discriminatory measures for identification, especially in instances where DNA has degraded and is unavailable or there is no reference sample to compare a

profile against. This will prove especially useful in cases of commingled remains. When compared to DNA processing, especially mitochondrial DNA, isotope analyses are quicker, less expensive, and much less vulnerable to contamination.

The results of this study will hopefully have wide-reaching effects across the medico-legal spectrum. This body of research will serve as the foundation for a database of modern, human, geolocational isotope values that will assist not only in the identification of fallen servicemen and women, but in the identification of victims of mass fatality incidents, undocumented aliens who perish attempting entry into the U.S., and local skeletal “Jane and John Doe” cases. This database will be available to the public free of charge and hopefully grow through future personal efforts and the contributions of other researchers.

APPENDIX A

REPLICATED VETERANS AFFAIRS BINDER

Stable Isotopes Research

Master Documents

Table of Contents

UNIVERSITY OF FLORIDA

Health Center Institutional Review Board

FWA00005790

PO Box 100173
Gainesville, Florida 32610-0173
Tel: (352) 846-1494
Fax: (352) 846-1497

MEMORANDUM

DATE: October 11, 2005

TO: Jack B. Meyer, DDS/DMD
VAMC 160 / 1601 SW Archer Road
Gainesville, FL 32608

FROM: Charles Riggs, M.D.
Vice Chairman, IRB - 01

EXPIRES: Thursday, September 21, 2006

SUBJECT: EXPEDITED IRB #474-2005

TITLE: EXPEDITED: ISOTOPIC DETERMINATION OF REGION OF ORIGIN IN MODERN PEOPLES: APPLICATION FOR IDENTIFICATION OF U.S. WAR - DEAD FROM THE VIETNAM CONFLICT

You have received IRB approval to conduct the above-listed research study. Approval of this study was granted on September 21, 2005. Enclosed is the dated, IRB-approved Informed Consent Form that must be used for enrolling subjects into this project from September 21, 2005 through September 20, 2006. This study is approved as expedited as it poses minimal risk and is approved under the following expedited category/categories:

Expedited #3: Prospective collection of biological specimens for research purposes by noninvasive means. Examples: (a) hair and nail clippings, if collected in a non-disfiguring manner; (b) deciduous teeth at time of exfoliation or if routine patient care indicates a need for extraction; (c) permanent teeth, if routine patient care indicates a need for extraction; (d) excreta and external secretions (including sweat); (e) uncannulated saliva collected either in an unstimulated fashion or stimulated by chewing gumbase or wax or by applying a dilute citric solution to the tongue; (f) placenta removed at delivery; (g) amniotic fluid obtained at the time of rupture of the membrane before or during labor; (h) supra- and sub-gingival dental plaque and calculus, provided the collection procedure is not more invasive than routine prophylatic scaling of the teeth and the process is accomplished in accordance with accepted prophylatic techniques; (i) mucosal and skin cells collected by buccal scraping or swab, skin swab, or mouth washings; (j) sputum collected after saline mist nebulization.

Expedited #7: Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies. Note: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR § 46.101(b) (2) and (b)(3). This listing refers only to research that is not exempt.

You are responsible for applying for renewal of this study prior to the expiration date. Re-approval of this study must be granted before the expiration date, or the study will automatically be suspended. If suspended, new subject accrual must stop. Research interventions must also stop unless there is a concern for the safety or well-being of the subjects. You MUST respond to the Continuing Review questions within 90 days or your study will be referred to the Board for termination.

The IRB has approved exactly what was submitted. Any change in the research, no matter how minor, may not be initiated without IRB review and approval, except where necessary to eliminate hazards to human subjects. If a change is required due to a potential hazard, that change must be promptly reported to the IRB.

If applicable, only a qualified clinician may be responsible for study-related healthcare decisions.

Any severe and unanticipated side effects or problems and all deviations from federal, state, university, or IRB regulations must be reported, in writing, within 5 working days.

Upon completion of the study, you are REQUIRED to submit a summary of the study and a Study Closure report to the IRB office.

Research records must be retained for 3 years after completion of the research; if the study involves medical treatment, it is recommended that the records be retained for 8 years.

If VAMC patients will be included in this study, or if the study is to be conducted in part on VA premises or performed by a VA employee during VA-compensated time, review by the VA Subcommittee for Clinical Investigations is required.

You are responsible for notifying all parties about the approval of this study, including your co-Investigators and Department Chair. If you have any questions, please telephone the IRB-01 office at (352) 846-1494.

cc: IRB file / Pharmacy / VA Research Center / Clinical Research Center

Department of Veterans Affairs

Memorandum

Date: October 19, 2005

From: Carsten Schmalfuss, M.D., Chairperson, Subcommittee for Clinical Investigation

Subj: "Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-Dead from the Vietnam Conflict" IRB #474-05

To: Jack Meyer, DDS/DMD (PI)

The Subcommittee for Clinical Investigation (SCI) reviewed the above protocol at its October 18, 2005 meeting. The Subcommittee has approved this protocol pending receipt of Investigator training documents for the following individuals:
Training required: "Overview of Good Clinical Practice and Human Participants Protection"

Jack Meyer, DDS/DMD
Laura A. Regan, MS/MA
Ray Berringer DMD/DDS

For specifics, please see page 2 of this memo or refer to the IRB website, sub-section VA http://irb.ufl.edu/irb01/othercommittees.htm#VA.

Research may begin following R&D Committee approval and completion of all training and staff clearance. A VA approval form (form 12-1223) will be sent to you as confirmation of VA Research and Development Committee approval. The next scheduled R&D Committee meeting will be held November 14, 2005.

If you have questions you may reach Tiffany at (352) 376-1611 ext. 5955.

CARSTEN SCHMALFUSS, M.D.

Cc: Research Office

Automated VA FORM 2105

Important Reminders:

1. **Health Center IRB approval is required:** (1) prior to initiating studies at the Gainesville VAMC; and (2) for all protocol/informed consent revisions. Please be sure that any modifications/additions/etc. submitted to the Health Center IRB are also submitted to the Gainesville VAMC for approval along with the appropriate cover form.
2. **Disclosures of PHI:** Studies with a HIPAA waiver of authorization require tracking of disclosures of Personal Health Information (PHI). A PHI tracking form is available at http://privacy.health.ufl.edu/policies/hipaamanual/forms/FF-OG-16.pdf.
3. **Adverse Events:** Please submit two copies of any Adverse Event or Adverse Drug Reaction to the VAMC Research Office.
4. **Drug Studies:** All investigational drugs administered or prescribed for patients and or non-patient volunteers within the confines of the Department of Veterans Affairs Medical Center must be dispensed by the VAMC Pharmacy Service.
5. **Witness Signature: The consent must also be signed and dated by a witness** whose role is to witness the subject's or the subject's legally authorized representative's signature and by the investigator or the person obtaining the informed consent.
6. **Medical Record Entry of Research Participation Required for VA subjects:** Investigators/Coordinators must have VA computer access to enter research information in the electronic medical record (CPRS) for all VA subjects. Contact the Human Resource Service (x.6009) for VA computer access and Research Service (x.5955) for CPRS access.
7. **Fees:** If your protocol is sponsored with funds through the North Florida Foundation for Research and Education (NFFRE), you may be exempt from the administrative protocol fee. Please contact Lisa at ext. 6477 within seven (7) days of receipt of this memo to request the waiver of fees.
8. **Training:** Each person involved in the study must complete an educational course or a web-based course on **both** the protection of human research subjects, as well as Good Clinical Practice (GCP). All investigators, research coordinators and research assistants involved in VA human studies research must receive appropriate training in the ethical principles and accepted practices on which human studies research should be conducted. The combined online Good Clinical Practice (GCP) and Human Participant Protection Education (HPPE) course, "Overview of GCP and Human Subjects Protection," satisfies the annual training requirement for research personnel in both GCP *and* human subjects' protection education. Please note that satisfactory completion of this course is required annual training. Annual training" is defined as fiscal year, i.e. 10/1/03-9/30/04. "Satisfactory completion" means that the individual scores 75% or above on each of the 9 module examinations. **VA Employees must register and complete this course on the Employee Education System On-Line Learning website at <http://vaww.ees.aac.va.gov>. Non-VA employees can register for this program at: <http://www.ees-learning.net>.** Co-Investigators, Sub-Investigators and Research Staff who do not work at the VA and do not come to the VA **to perform research and/or to enroll VA patients** do not have to complete the human subject protection training or the Good Clinical Practice training however, the Co-I, Sub-I, or other Research staff member may be required to do HIPAA training if s/he has access to VA Personal Health Information (PHI). The VHA/HIPAA Privacy Training Course can be accessed at **www.VHAprivacytraining.net** or **www.vhaprivacytraining.med.va.gov** ; the HIPAA training is not required **IF** the expectation is that VHA PHI will not be accessed, but if the potential for accessing VHA PHI is great, e.g. analyzing tests that include VHA patient identifiers, or providing emergency oversight to the research study because the PI is unavailable or on extended leave, the staff member should complete the privacy training course at that time. A certificate of training for each Co-I, Sub-I or other staff member, or written documentation that the staff member does not have VA involvement is required in Research before any project is initiated. **Please note that the Co-I, Sub-I or other staff member will be required to complete the training later if they want to change their level of involvement with VA subjects.**

   In addition to the above education, all non-M.D. Investigators, study coordinators, and research assistants (including WOC's who perform independent clinical activities) must provide: (1) verification of their licensure and education (2) a scope of practice or duties; and (3) must apply for a "Without Compensation" clearance if they are not VA employees. If you do not have a scope of practice you may send Position Description (PD) or functional statement.

Enclosed is the VA Research and Development Committee approval (form 10-1223) for your study. If you have questions please contact me at 376-1611 x. 5955 or email me at *Tiffany.Ramsey@med.va.gov* .

Thank you.

TIFFANY RAMSEY
Program Support Assistant for Research

Department of Veterans Affairs

REPORT OF SUBCOMMITTEE ON HUMAN STUDIES

VA #0001

PROJECT/PROGRAM TITLE:
"Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-Dead from the Vietman Conflict"

PRINCIPAL INVESTIGATOR:
Jack Meyer, DMD

VAMC:	REVIEW DATE:
Gainesville, Florida	9/21/05 IRB #474-05

COMMITTEE FINDINGS:

1. The information given in the Informed Consent under the Description of Research by Investigator is complete, accurate, and understandable to a research subject or a surrogate who possesses standard reading and comprehension skills. ☒ YES ☐ NO

2. The informed consent is obtained by the principal investigator or a trained and supervised designate under suitable circumstances. ☒ YES ☐ NO

3. Every effort has been made to decrease risk to subject(s)? ☒ YES ☐ NO

4. The potential research benefits justify the risk to subject(s)? ☒ YES ☐ NO

5. If subject is incompetent and surrogate consent is obtained, have all of the following conditions been met; a) the research can't be done on competent subjects; b) there is no risk to the subject, or if risk exists the direct benefit to subject is substantially greater; c) if an incompetent subject resists, he will not have to participate; d) if there exists any question about the subject's competency, the basis for decision on competency has been fully described. ☒ YES ☐ NO

6. If the subject is paid the payment is reasonable and commensurate with the subject's contribution. ☒ YES ☐ NO

7. Members of minority groups and men have been included in the study population whenever possible and scientifically desirable. ☒ YES ☐ NO

8. Comments: (Indicate if Expedited Review Review)
This Expedited study was reviewed and approved by the Research & Development Committee on November 14, 2005. IRB Protocol version 03.09.04 is approved for use.

RECOMMENDATION: ☒ APPROVE ☐ DISAPPROVE / REVISE

SIGNATURE OF CHAIRMAN	DATE
	11/14/05

VA FORM
OCR 1995(R) **10-1223**

DEPARTMENT OF THE AIR FORCE

HEADQUARTERS UNITED STATES AIR FORCE ACADEMY

USAF ACADEMY COLORADO

MEMORANDUM FOR MAJ OUELLETTE 22 July 2005

FROM: HQ USAFA/XPX

SUBJECT: Protocol FAC2005026H Approved

1. The HQ USAFA Institutional Review Board considered your protocol FAC2005026, Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-dead from the Vietnam Conflict at its 21 July 2005 meeting. The study was approved as exempt from IRB oversight in accordance with *32 CFR 219.101, paragraph (b)(2).* The board agreed that sufficient safeguards were in place to protect research participants and deemed this study can be exempt; you will not need to use the ICD or HIPPA Forms. Please place the following statement at the bottom of your recruitment material: 'Approved: HQ USAFA IRB FAC2005026H.' This will inform potential subjects that your research has been reviewed and approved. Please note that the USAFA Authorized Institutional Official, HQ USAFA/DS and the Biomedical Research and Compliance Office of the Surgeon General's Office, AFMSA/SGRC review all USAFA IRB actions and may amend this decision or identify additional requirements.

2. The protocol will be considered closed, but will be retained in XPX for 5 years then sent to permanent storage. As the principal investigator on the study, the Biomedical Research and Compliance Office requires that you retain your data, reports, etc. for 3 years following completion of the study.

3. If the conditions under which you have been granted exempt status change, you must notify the IRB Chair or IRB Administrator immediately. We will advise you on whether additional IRB review is required

4. If you have any questions or if I can be of further assistance, please don't hesitate to contact me at 333-3091 or the IRB Chair, Dr. Kate Carson at 333-2597.

Kathleen A. O'Donnell

KATHLEEN A. O'DONNELL, PhD
HQ USAFA IRB Administrator

JPAC Isotope Study
Dental Staff Instructions

Thank you for your assistance with this worthwhile project. The purpose of this study is to determine if stable isotopes serve as geographic markers for regions of childhood residency. Patients, to include cadets, active-duty, retirees, and dependents, already identified by the 10 DS for tooth extraction will be asked to participate in a brief survey and donate their extracted teeth for analysis. The investigators will be examining the mineral elements in human tooth enamel. Initial efforts will focus on carbon, oxygen, strontium, and lead stable isotope ratios. No DNA analysis will be performed. The survey information will be used to help determine if geographic regions of the United States have specific isotopic signatures that become incorporated into dental tissues. This study will also assess if these isotopic signatures vary through time. When compared to isotopic signatures developed for geographic areas of Southeast Asia, it is hoped the information will assist in identifying the origin of unknown dental remains unilaterally turned over to, or recovered by, the Joint POW/MIA Accounting Command (JPAC) Central Identification Laboratory. Additionally, this information may be applied to identification efforts of fallen servicemen and women in conflicts outside of Southeast Asia and in the identification of victims of mass disasters such as airliner crashes and the events of 11 September 2001. A total of 100-200 participants will be surveyed from this facility. A further 200-300 patients will be sampled following the same protocol from the 10th Dental Squadron, U.S. Air Force Academy. Participant involvement is limited to tooth donation and completion of the JPAC Central Identification Laboratory survey.

If you would like more information about this project, see the Isotope Study Information document provided for participants' information. Please familiarize yourself all the necessary paperwork associated with this study and with these instructions. If you have any questions please contact Dr Jack Meyer or Dr Ray Berringer, at 379-4040, or Maj Laura Regan 392-6772.

<u>Instructions</u>

- Please query all patients scheduled for dental extractions of <u>permanent teeth</u> as to their willingness to participate in this study. We need as large and broad of a sample population as possible. This is best done as soon as the patients are notified their teeth will be pulled. This will give them time to consider participating prior to enrollment in the study.
    - Minors, prisoners, UF students, and those with diminished mental capacity **will not** be included in this study.
    - Please emphasize to the patient that nonparticipation or voluntary withdrawal from the investigation cannot, and will not, be the basis for any retribution brought against the subject.

- If the patient would like additional information, please provide them with a copy of the study information question and answer material provided with this package.
- At patient intake/inprocessing, completion of two forms is required of all subjects:
    1. *VA Research Consent Form* (sign two copies)
        - This is a combined Informed Consent and Health Insurance Portability & Privacy Act (HIPAA) form specific to this study and separate from any forms that may need to be administered for routine care.
        - The dental staff member must sign last page of the form as the "Person Obtaining Consent and Authorization" (1^{st} blank)
        - Volunteer/participant needs to read form, fill in their name in both locations on the front page and **sign** the back page **in front of a witness** as the "Person Consenting and Authorizing" (2^{nd} blank)
        - Allow the volunteer as much time as needed to read the form.
        - Witness signs last page of form (3^{rd} and last blank) verifying volunteer's signature
        - Each individual needs to sign two original forms.
        - After all three individuals have signed, the volunteer/participant **<u>must</u>** be given one of the copies of the form prior to participation in the study. The second hard copy needs to be filed in the study master document binder.
    2. *JPAC Survey with map*
        - Volunteer/participant needs to read form and answer all questions including drawing the numbers associated with where they lived as children on the map. (question #7)
        - Once completed, please go over the information with the patient to ensure it is all accurate. Read the questions and answers to the patient affirming the answers provided are correct. Check the map for completion.
        - An example of a completed survey is provided for guidance to staff and patients. Please make it available to patients should they desire it.
- Retain all forms. A binder will be provided for storage of the original VA Research Consent Forms and the JPAC Isotope Study Subject Identifier Key. The survey form should be maintained in the patient's record until the date of extraction.
- Just prior to extraction, assign each individual a unique study identifier utilizing the JPAC Isotope Study Subject Identifier Key.
    - **It is critical that no numbers are repeated** so completely fill in the log as numbers are assigned. The first person assigned to the study should have number VA-001, the second person volunteering for the study should be VA-002, and so on.
    - Prior to assigning a number, check question #8 on the survey. If a subject has not had a dental extraction performed by your facility within the last year (i.e., answered "No") then assign them the next number in the log. If they answered "Yes," you need to go back in their record and cross-check

it with the log to find out what their original number was and give them the same number, except add on a “b” suffix. So if someone was assigned identifier VA-123 for their first extraction, their second visit would be VA-123b.

  - All teeth extracted on the same day will have the same identifier number. **The <u>number is associated with the individual, not each tooth</u>.**

- Write the sample identifier number on the member’s survey on the appropriate blank in the gray “For Dental Staff Use Only” box.
- If multiple teeth are being pulled, collect as many teeth as possible from each individual.
- Write the tooth number (position in the arcade) for all teeth donated and the date of extraction on the appropriate blank on the member’s survey in the gray “For Dental Staff Use Only” box.
- Write the sample identifier and tooth number on the storage vial with a Sharpie or other permanent marker. Only place one tooth into each vial.
- Write the sample identifier on the plastic pouch with a Sharpie or other permanent marker. Place all of the tooth vials in the plastic pouch, seal the pouch, and staple the pouch to the volunteer/participant survey. All vials, the pouch, and the survey should have the same sample identifier.
- Dr Meyer will identify a container for storage of the associated teeth and surveys.
- The surveys and associated teeth will be picked up by Major Laura Regan. If you need to contact her for some reason, her information is as follows:

  Laura Regan
  C.A. Pound Human Identification Laboratory
  University of Florida
  Bldg 114, Radio Road
  Gainesville FL 32611-2545
  Phone: 392-6772
  Fax: 392-2071

- This study is scheduled to end 1 Aug 2006.
- At the completion of the study, Major Regan will pick up all forms and arrange for final disposition.

Isotope Study

CPRS/VISTA Instructions

All research participation within the VA medical system must me documented in the subject's electronic medical record (CPRS). This action complies with federally mandated standards regarding research documentation. The electronic document eliminates the need to file the Informed Consent form in the subject's paper medical record. The template must be added to CPRS on the date the subject signs the Informed Consent document. Since subject participation is limited to a single office visit, the research flag must be removed from the system within one week of dental extraction(s).

<u>To Enter Patient in Study:</u>

- Open CPRS
- Select the patient's name
- Click on the "Notes" tab on the bottom toolbar
- On the left sidebar, click on "New Notes" or under the top "Action" tab, click on "New Progress Note"
- In the Location for Current Activity window, type in in "**GHIS**" (for GHistorical) in the visit location box **and** check the small right-hand box for historical visit
- Click "OK"
- In the title box, type in "**Dental Research-W (T)**"
- Click "OK"
- The IRB number is **474-05**
- Click "OK"
- On the left sidebar, click on "Templates"
- Under the "My Templates" file, select "Dental Isotope Study" (you may need to click on the "+" sign to reveal)
- Sign the note per normal CPRS procedures

The patient now has a clinical warning research alert

<u>To Remove Patient from Study/Remove Research Alert:</u>

- Log into VISTA
- At the *Select Dental/Control Point Official Menu Option* prompt, type in "**^TIU**" and press enter
- When it asks, *Would you like to resume editing now?*, type in "**No**" and press enter
- At the *Progress Notes User Menu* prompt, Type in "**1**" for "Select progress notes/discharge summary" and press enter
- At the *Progress Notes User Menu Option* prompt, type in "**2**" to review progress notes by patient and press enter
- Type in the patients name (last name, first) and select
- There may be a pause at this point while the system performs a means test
- If the patient was entered in the study today, type "**T**" and press enter, otherwise type in "T-(number of days ago patient was entered in study)"
- Select the number of the record with the "Dental Research-W (T)" warning title
- At the *Select Action* prompt, type "**CT**" and press enter
- You should receive a *Dental Research-W (T)* prompt; type in "**COMPLETED**" (all in caps) and press enter
- Exit VISTA

If you go back into CPRS, the template should now read "Dental Research Completed"

JPAC Isotope Study
Subject Identifier Key

	Extraction Date	Attending Dentist	Tooth Position	Study Identifier
Example	7 July 2005	Dr Berringer	1,16,17,32	**VA-000**
1				**VA-001**
2				**VA-002**
3				**VA-003**
4				**VA-004**
5				**VA-005**
6				**VA-006**
7				**VA-007**
8				**VA-008**
9				**VA-009**
10				**VA-010**
11				**VA-011**
12				**VA-012**
13				**VA-013**
14				**VA-014**
15				**VA-015**
16				**VA-016**
17				**VA-017**
18				**VA-018**
19				**VA-019**
20				**VA-020**
21				**VA-021**
22				**VA-022**
23				**VA-023**
24				**VA-024**
25				**VA-025**

Department of Veterans Affairs | **VA RESEARCH CONSENT FORM**

Subject Name: ______________________ Date ______

Title of Study: Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-Dead from the Vietnam Conflict.

Principal Investigator: Jack B. Meyer, Jr., DMD VAMC: NF/SG VHS

IRB # 474-2005

Informed Consent to Participate in Research and Authorization for Collection, Use, and Disclosure of Protected Health Information

University of Florida
Health Center
Institutional Review Board
APPROVED FOR USE
From 9/21/05 Through 9/20/06
CJD

You are being asked to take part in a research study. This form provides you with information about the study and seeks your authorization for the collection, use and sharing of your protected health information necessary for the study. The Principal Investigator (the person in charge of this research) or a representative of the Principal Investigator will also describe this study to you and answer all of your questions. Your participation is entirely voluntary. Before you decide whether or not to take part, read the information below and ask questions about anything you do not understand. If you choose not to participate in this study you will not be penalized or lose any benefits to which you would otherwise be entitled.

1. **Name of Participant ("Study Subject")**

   ____

2. **Title of Research Study**

   Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-Dead from the Vietnam Conflict.

3. **Principal Investigator and Telephone Number(s)**

   Dr. Jack B. Meyer, Jr. (352) 379-4040

   Contact information for emergencies after hours or on weekends or holidays: Laura Regan (352) 262-5153

Department of Veterans Affairs | **VA RESEARCH CONSENT FORM**

UF-IRB-01 SEP 21 2005 APPROVED

Subject Name: Date

Title of Study: Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-Dead from the Vietnam Conflict.

Principal Investigator: Jack B. Meyer, Jr., DMD VAMC: NF/SG VHS

4. Source of Funding or Other Material Support

University of Florida

5. What is the purpose of this research study?

The purpose of the study is to determine if the minerals in tooth enamel can be used to tell where a person grew up. You are asked to complete a brief survey and donate your pulled teeth for analysis. We will initially focus our efforts on carbon, oxygen, strontium, and lead stable isotope values. We will not be looking at DNA. The survey information will be used to help match mineral values in teeth to specific regions of the United States. These mineral values will first be compared to stable isotope (mineral) values for geographic areas of Southeast Asia. It is hoped the information will assist in identifying if unknown dental remains turned over to, or recovered by, the Joint POW/MIA Accounting Command (JPAC) Central Identification Laboratory are American or not. If the remains are of a U.S. MIA serviceman, the survey data will then allow us to narrow down where that individual grew up in the United States. Additionally, this information may be applied to identification efforts of fallen servicemen and women in conflicts outside of Southeast Asia and in the identification of victims of mass disasters such as airliner crashes and the events of 11 September 2001. We plan to survey 100-200 participants from this dental clinic. A further 100-200 patients will be sampled following the same protocol from the U.S. Air Force Academy, Oral Surgery Clinic. Your involvement is limited to tooth donation and completion of the JPAC Central Identification Laboratory survey.

6. What will be done if you take part in this research study?

If you volunteer to participate in this study, we will ask you to undergo the following procedures. You will complete a one-time JPAC Central Identification Laboratory survey to collect information concerning childhood residence and factors that affect permanent tooth formation and growth. You are also requested to donate all extracted teeth to the project for elemental analyses.

This study is independent of your scheduled dental procedure and will in no way affect the treatment you receive.

If you have any questions now or at any time during the study, you may contact the Principal Investigator listed in #3 of this form.

Department of Veterans Affairs

VA RESEARCH CONSENT FORM

UF-IRB-01 SEP 21 2005 APPROVED

Subject Name: Date

Title of Study: Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-Dead from the Vietnam Conflict.

Principal Investigator: Jack B. Meyer, Jr., DMD VAMC: NF/SG VHS

7. If you choose to participate in this study, how long will you be expected to participate in the research?

Participation should take 15-30 minutes to complete the necessary paperwork.

8. How many people are expected to participate in this research?

A total of 200-400 live subjects are expected to participate in this research. This includes 100-200 people here and 100-200 people from the U.S. Air Force Academy.

9. What are the possible discomforts and risks?

There are no known risks associated with this study.
This study may include risks that are unknown at this time.

Participation in more than one research study or project may further increase the risks to you. Please inform the Principal Investigator (listed in #3 of this consent form) or the person reviewing this consent with you before enrolling in this or any other research study or project.

Throughout the study, the researchers will notify you of new information that may become available and might affect your decision to remain in the study.

If you wish to discuss the information above or any discomforts you may experience, you may ask questions now or call the Principal Investigator or contact person listed on the front page of this form.

10a. What are the possible benefits to you?

There are no direct benefits to you for your participation in this study.

10b. What are the possible benefits to others?

The data generated from this study will assist in determining if a set of remains are of a U.S. serviceman or woman or someone who was raised in Southeast Asia. It is hoped that U.S. remains will be identified to a regional level. This project has the potential to assist in the identification of remains returned from Southeast Asia and bring closure to the disappearance of a loved one or friend. Additionally, this information may be applied to identification efforts of fallen servicemen and women in conflicts outside of

Department of Veterans Affairs

VA RESEARCH CONSENT FORM

UF-IRB-01 SEP 21 2005 APPROVED

Subject Name: Date

Title of Study: Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-Dead from the Vietnam Conflict.

Principal Investigator: Jack B. Meyer, Jr., DMD VAMC: NF/SG VHS

Southeast Asia and in the identification of victims of mass disasters such as airliner crashes and the events of 11 September 2001.

11. If you choose to take part in this research study, will it cost you anything?

There is no charge to you associated with your participation in this study.

Costs for routine medical care procedures that are not being done only for the study will be charged to you or your insurance. These costs may not be charged if you are a veteran and you are being treated at the North Florida/South Georgia Veterans Health System (NF/SG VHS).

12. Will you receive compensation for taking part in this research study?

You will not be compensated for taking part in this study.

13. What if you are injured because of the study?

If you experience an injury that is directly caused by this study, only professional dental care that you receive at the University of Florida Health Science Center will be provided without charge. However, hospital expenses will have to be paid by you or your insurance provider. No other compensation is offered. Please contact the Principal Investigator listed in Item 3 of this form if you experience an injury or have any questions about any discomforts that you experience while participating in this study.

You will not have to pay hospital expenses if you are being treated at the North Florida/South Georgia Veterans Health System (NF/SG VHS) and experience any physical injury during participation in a Veterans Health System-approved study.

14. What other options or treatments are available if you do not want to be in this study?

The option of taking part in this study is doing nothing. If you do not want to take part in this study, tell the Principal Investigator or his/her assistant and do not sign this Informed Consent Form. Choosing not to participate will in no way affect your normally scheduled dental procedure.

Department of Veterans Affairs

VA RESEARCH CONSENT FORM

Subject Name: Date

Title of Study: Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-Dead from the Vietnam Conflict.

Principal Investigator: Jack B. Meyer, Jr., DMD VAMC: NF/SG VHS

15a. Can you withdraw from this research study?

You are free to withdraw your consent and to stop participating in this research study at any time until creation of the secure database. Once the database has been completed and your teeth have been sampled however, we will be unable to trace your information back to you and withdraw you from the study. If you do withdraw your consent, there will be no penalty, and you will not lose any benefits you are entitled to.

If you decide to withdraw your consent to participate in this research study for any reason, you should contact Laura Regan at (352) 392-6772.

If you have any questions regarding your rights as a research subject, you may phone the Institutional Review Board (IRB) office at (352) 846-1494.

15b. If you withdraw, can information about you still be used and/or collected?

If you withdraw from this study prior to sampling of your teeth, neither your teeth nor your survey information will be used. After this time we will not be able to identify a survey or teeth as belonging to you.

15c. Can the Principal Investigator withdraw you from this research study?

You may be withdrawn from the study without your consent for the following reasons:

- Survey data is incomplete.
- There is not enough enamel on your tooth or teeth to provide an adequate sample for analysis.

16. If you agree to participate in this research study, the Principal Investigator will create, collect, and use private information about you and your health. Once this information is collected, how will it be kept secret (confidential) in order to protect your privacy?

Information collected about you and your health (called protected health information), will be stored in locked filing cabinets or in computers with security passwords. Only certain people have the legal right to review these research records, and they will protect the secrecy (confidentiality) of these records as much as the law allows. These people include the researchers for this study, certain University of Florida officials, the hospital or clinic (if any) involved in this research, and the Institutional Review Board (IRB; an IRB is a group of people who are responsible for looking after the rights and welfare of people taking part in research).

Otherwise your research records will not be released without your permission unless required by law or a court order.

If you participate in this research study, the researchers will collect, use, and share your protected health information with others. Items 17 to 26 below describe how this information will be collected, used, and shared.

17. If you agree to participate in this research study, what protected health information about you may be collected, used and shared with others?

Your protected health information may be collected, used, and shared with others to determine if you can participate in the study, and then as part of your participation in the study. This information can be gathered from you or your past, current or future health records, from procedures such as physical examinations, x-rays, blood or urine tests or from other procedures or tests. This information will be created by receiving study treatments or participating in study procedures, or from your study visits and telephone calls. More specifically, the following information may be collected, used, and shared with others:

- Date of birth
- Sex
- Race
- Tobacco use
- Childhood diet
- History of residence until age 18
- Previous history of tooth extraction within the last year
- Stable isotope analyses findings

If you agree to be in this research study, it is possible that some of the information collected might be copied into a "limited data set" to be used for other research purposes. If so, the limited data set may only include information that does not directly identify you. For example, the limited data set cannot include your name, address, telephone number, social security number, or any other photographs, numbers, codes, or so forth that link you to the information in the limited data set. If used, limited data sets have legal agreements to protect your identity and confidentiality and privacy.

Department of Veterans Affairs | VA RESEARCH CONSENT FORM

SF-IRB-02 SEP 21 2005 APPROVED

Subject Name: ______________________ Date ______________

Title of Study: Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-Dead from the Vietnam Conflict.

Principal Investigator: Jack B. Meyer, Jr., DMD VAMC: NF/SG VHS

18. For what study-related purposes will your protected health information be collected, used, and shared with others?

Your protected health information may be collected, used, and shared with others to make sure you can participate in the research, through your participation in the research, and to evaluate the results of the research study. More specifically, your protected health information may be collected, used, and shared with others for the following study-related purpose(s):

- to associate specific isotope ratios with particular locations of residence
- to determine if isotope ratios are affected by factors such as age, sex, race, and tobacco use
- to evaluate dietary influence on isotope ratios

19. Who will be allowed to collect, use, and share your protected health information?

Your protected health information may be collected, used, and shared with others by:

- the study Principal Investigator Jack Meyer, Jr., DMD, and his staff
- Malcolm Randall VA Medical center (Gainesville)
- the University of Florida Institutional Review Board
- the U.S. Air Force Academy or Department of Defense representatives
- Joint POW/MIA Accounting Command, Hickam Air Force Base, HI
- C.A. Pound Human Identification Laboratory, University of Florida

20. Once collected or used, who may your protected health information be shared with?

Your protected health information may be shared with:

- the study sponsor, the C.A. Pound Human Identification Laboratory, University of Florida
- other professionals at the University of Florida or Shands Hospital that provide study-related treatment or procedures
- Joint POW/MIA Accounting Command Central Identification Laboratory
- United States and foreign governmental agencies who are responsible for overseeing research, such as the Food and Drug Administration, the Department of Health and Human Services, and the Office of Human Research Protections
- Government agencies who are responsible for overseeing public health concerns such as the Centers for Disease Control and Federal, State and local health departments

Department of Veterans Affairs

VA RESEARCH CONSENT FORM

Subject Name: **Date**

Title of Study: Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-Dead from the Vietnam Conflict.

Principal Investigator: Jack B. Meyer, Jr., DMD **VAMC:** NF/SG VHS

21. If you agree to participate in this research, how long will your protected health information be used and shared with others?

Hard copies of the survey information will be maintained by the associate investigator for 5 years. Data will also be maintained indefinitely by the JPAC Central Identification Laboratory in the form of a secure database utilized for comparative purposes. Individualizing information such as name and social security number will not be collected and hence information in the database will not be able to be traced back to you.

If you withdraw your permission for the use and sharing of your protected health information after the database has been created, we will not be able to identify which survey and tooth sample belonged to you.

22. Why are you being asked to allow the collection, use and sharing of your protected health information?

Under a new Federal Law, researchers cannot collect, use, or share with others any of your protected health information for research unless you allow them to by signing this consent and authorization.

23. Are you required to sign this consent and authorization and allow the researchers to collect, use and share with others your protected health information?

No, and your refusal to sign will not affect your treatment, payment, enrollment, or eligibility for any benefits outside this research study. *However, you cannot participate in this research unless you allow the collection, use and sharing of your protected health information by signing this consent/authorization.*

24. Can you review or copy your protected health information that has been collected, used or shared with others under this authorization?

You have the right to review and copy your protected health information. However, you will not be allowed to do so until after the study is finished.

25. Is there a risk that your protected health information could be given to others beyond your authorization?

Yes. There is a risk that information received by authorized persons could be given to others beyond your authorization and not covered by the law.

Department of Veterans Affairs

VA RESEARCH CONSENT FORM

UF-IRB-01 SEP 21 2005 APPROVED

Subject Name: ____________________ Date ____________

Title of Study: Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-Dead from the Vietnam Conflict.

Principal Investigator: Jack B. Meyer, Jr., DMD VAMC: NF/SG VHS

26. Can you revoke (cancel) your authorization for collection, use and sharing with others of your protected health information?

Yes. You can revoke your authorization at any time before, during, or after your participation in the research. If you revoke, no new information will be collected about you. However, information that was already collected may still be used and shared with others if the researchers have relied on it to complete and protect the validity of the research. You can revoke your authorization by giving a written request with your signature on it to the Principal Investigator.

27. How will the researcher(s) benefit from your being in this study?

In general, presenting research results helps the career of a scientist. Therefore, the Principal Investigator may benefit if the results of this study are presented at scientific meetings or in scientific journals.

Department of Veterans Affairs | **VA RESEARCH CONSENT FORM**

Subject Name: Date

Title of Study: Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-Dead from the Vietnam Conflict.

Principal Investigator: Jack B. Meyer, Jr., DMD VAMC: NF/SG VHS

28. Signatures

As a representative of this study, I have explained to the participant the purpose, the procedures, the possible benefits, and the risks of this research study; the alternatives to being in the study; and how the participant's protected health information will be collected, used, and shared with others:

Signature of Person Obtaining Consent and Authorization Date

You have been informed about this study's purpose, procedures, possible benefits, and risks; the alternatives to being in the study; and how your protected health information will be collected, used and shared with others. You have received a copy of this Form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time.

You voluntarily agree to participate in this study. You hereby authorize the collection, use and sharing of your protected health information as described in sections 17-26 above. By signing this form, you are not waiving any of your legal rights.

Signature of Person Consenting and Authorizing Date

VA regulations require a witness for all of the signatures provided above.

Signature of Witness Date

For a copy of a blank survey see Figure 4-1.

Joint POW/MIA Accounting Command
JPAC
"Until they are home"

Thank you for your participation in this study. Its purpose is to provide a powerful new tool to assist in identifying our fallen servicemen and women. The information you provide will be used to determine if geographic regions of the U.S. have specific isotopic signatures that become incorporated into dental tissues. We will be looking at the mineral elements in your teeth. No DNA analysis will be performed. When compared with isotopic signatures developed for geographic areas of Southeast Asia, it is hoped the information will identify the origin of unknown remains recovered by JPAC's Central Identification Laboratory. Additionally, the information gleaned from this study may prove useful in identifying remains recovered from further conflicts such as World War II and encountered in mass disasters such as airliner crashes and the events of 11 September 2001.

Instructions: Please fully answer all **8** questions. Incomplete data may exclude your teeth from the study. If you have any questions, please ask your attending dental staff.

For Dental Staff Use Only

Sample identifier ______________

Position(s) in arcade ______________

Date of extraction ______________

1) Date of birth (day/month/year) __01/Dec/1953 _______

2) Sex (circle one) **Male** **Female**

3) What race do you consider yourself? ____White_______

4) Have you ever regularly used tobacco products (i.e. cigarette, chew, snuff)? **Yes** **No**

If yes, what products did/do you use, what was the time period, and what was the frequency (i.e. 1 pack a day)?

	Tobacco Product	Used From (year)	Used Until (year)	Frequency
1.	cigarettes	1971	Present	½ pack a day
2.	chew	1980	1988	4 times a day
3.				

5) Which of the following categories would you consider your childhood diet to the age of 18. Please circle only one category unless you had a major diet shift. If so, please indicate the ages at which you followed each diet.

Meat Eater **Vegetarian** **Vegan**

6) What locations have you lived in, starting with birth and extending to age 18? Please be as specific as possible. If you require more room, please use the back of this sheet.

	From (year)	To (year)	City	State	Country
1.	1953	1954	Seattle	WA	USA
2.	1954	1956	Frankfurt		Germany
3.	1956	1971	Omaha	NE	USA
4.					
5.					
6.					
7.					
8.					
9.					
10.					

7) Please indicate the approximate location of each of the above areas on the attached map. For area (1) write a ①, area (2) write a ②, etc. If you lived outside of the U.S. for any portion of your childhood, please disregard for the extent of your domicile outside of the U.S. Do include the numbers for any corresponding time lived in the U.S.

8) Have you undergone any prior dental extractions at this facility within the past year? (circle one) **Yes** **No**

If yes, please indicate the date to the best of your recollection. (day/month/year) ______________

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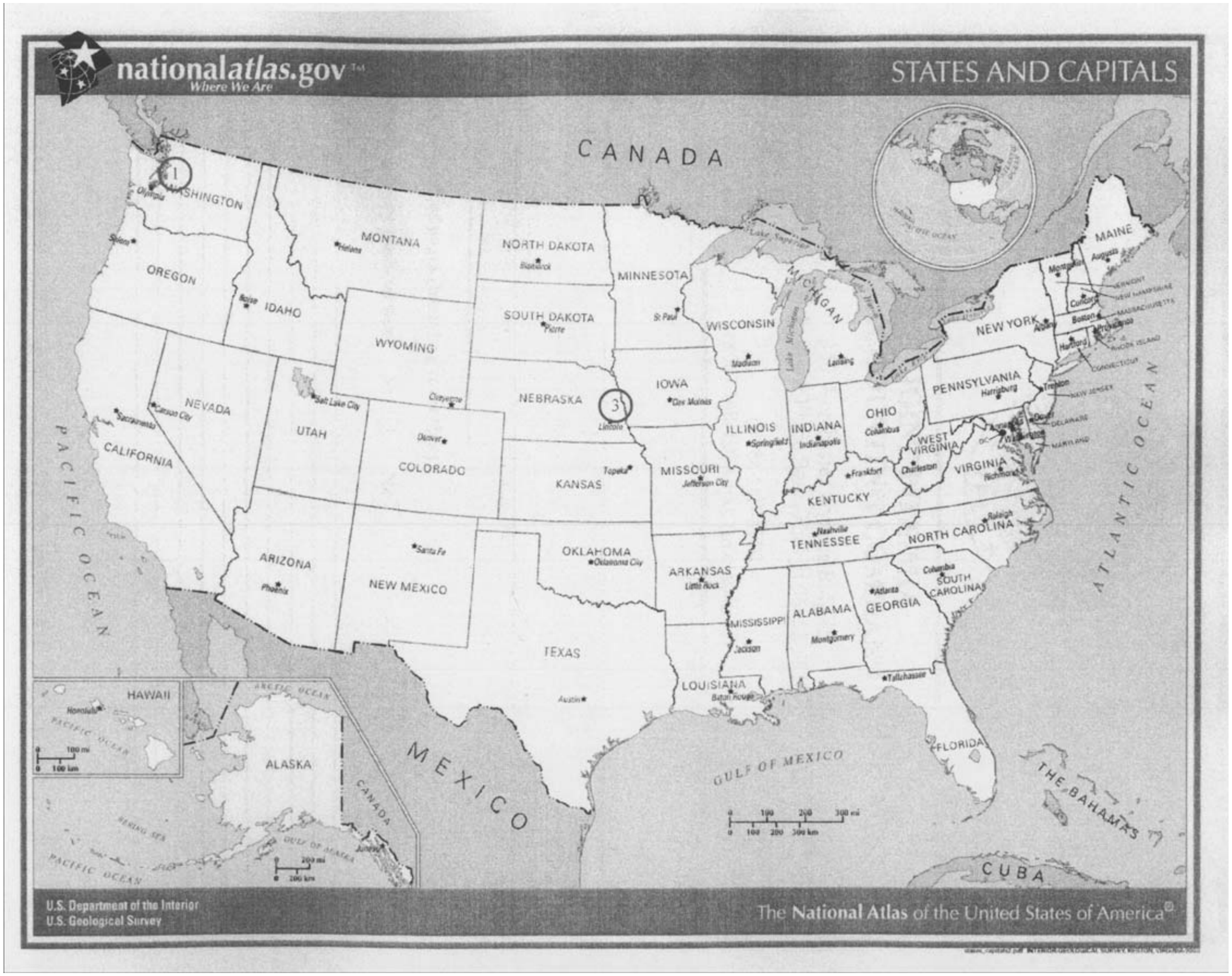
nationalatlas.gov
Where We Are
STATES AND CAPITALS
CANADA
WASHINGTON
Olympia
OREGON
Salem
MONTANA
Helena
IDAHO
Boise
NORTH DAKOTA
Bismarck
SOUTH DAKOTA
Pierre
MINNESOTA
St Paul
WYOMING
Cheyenne
WISCONSIN
Madison
MICHIGAN
Lansing
NEW YORK
Albany
MAINE
Augusta
Montpelier
Concord
Boston
Providence
Hartford
NEBRASKA
Lincoln
IOWA
Des Moines
NEVADA
Carson City
UTAH
Salt Lake City
CALIFORNIA
Sacramento
COLORADO
Denver
KANSAS
Topeka
MISSOURI
Jefferson City
ILLINOIS
Springfield
INDIANA
Indianapolis
OHIO
Columbus
PENNSYLVANIA
Harrisburg
Trenton
WEST VIRGINIA
Charleston
VIRGINIA
Richmond
KENTUCKY
Frankfort
TENNESSEE
Nashville
NORTH CAROLINA
Raleigh
SOUTH CAROLINA
Columbia
ARIZONA
Phoenix
NEW MEXICO
Santa Fe
OKLAHOMA
Oklahoma City
ARKANSAS
Little Rock
MISSISSIPPI
Jackson
ALABAMA
Montgomery
GEORGIA
Atlanta
Tallahassee
FLORIDA
TEXAS
Austin
LOUISIANA
Baton Rouge
PACIFIC OCEAN
ATLANTIC OCEAN
GULF OF MEXICO
MEXICO
THE BAHAMAS
CUBA
HAWAII
Honolulu
ALASKA
Juneau
CANADA
1
3
U.S. Department of the Interior
U.S. Geological Survey
The National Atlas of the United States of America

ISOTOPE STUDY INFORMATION

Q. ***Why is this study being conducted?***

A. Our goal is to create a geographic fingerprint utilizing carbon, oxygen, strontium, and lead isotope ratios for modern people that will pinpoint the region of origin for unknown skeletal material. If successful, this information will allow us to distinguish the remains of people who grew up in Southeast Asia from those who were raised in the U.S. Stable isotopes will allow us to further narrow down where someone grew up in the U.S. to the regional level, boosting discriminatory power. Our efforts will initially focus on the roughly 1,800 service members who remain unaccounted for from the Vietnam conflict (JPAC) and hopefully expand to encompass all previous wars from which we are still recovering the remains of our fallen heroes.

Q. ***Who is supporting this study?***

A. This project is sponsored by the Joint POW/MIA Accounting Command's (JPAC's) Central Identification Lab, Hickam Air Force Base, and the C.A. Pound Human Identification Laboratory, University of Florida. The 10th Dental Squadron, United States Air Force Academy, and the North Florida/South Georgia Veterans Health System Dental Service, Department of Veterans Affairs, are acting as liaisons with patients for donation of their teeth.

Q. ***What are stable isotopes?***

A. Stable isotopes are nonradioactive atoms whose nuclei contain the same number of protons while differing in their number of neutrons (Hoefs 2004). The atomic mass of different isotopes of the same element will vary because the atomic mass is a measure of the sum of the number of protons and neutrons (Hoefs 2004). So while isotopes of a like element will react the same chemically, they will react at different rates due to their different atomic masses. Different metabolic and chemical processes therefore, change the ratios between the isotopes in a characteristic manner (van der Merwe 1985). When these ratios are compared against known industry standards using a mass spectrometer, conclusions can be drawn pertaining to a specific set of questions. For example, carbon has three isotopes: ^{12}C, ^{13}C, and ^{14}C. The first two are stable isotopes, while the latter is radioactive (van der Merwe 1985). A carbon atom with a mass of 13 (denoted ^{13}C) has one more neutron than a carbon atom with a mass of 12 (^{12}C). These different isotopes are taken up in different amounts and at different rates and deposited in body tissues during digestive processes.

Q. ***What do the isotopes analyzed for this study tell us?***

A. Carbon

Carbon isotope ratios depend on cultural food preferences. The different photosynthetic pathways of plants produce varying carbon isotope values. C_3 photosynthesis occurs in plants such as wheat, rice, and barley, and produces a three-carbon compound while C_4 photosynthesis, found in sugar cane and corn, produces a 4-carbon acid (MacFadden *et al* 1999). These different photosynthetic processes produce different isotopic ratios, which are then incorporated into plant tissues. What makes carbon isotope analyses so powerful is that the ranges of C_3 and C_4 plants do not overlap. Because of this, carbon signatures should be significantly different between a traditional, rice-based, Vietnamese diet versus a sugary, wheat- and corn-based diet common to Americans growing up in the 1950s-1960s.

Oxygen

Oxygen isotopes found in the body come from local water sources and are a reflection of a particular environment or geographic region. Oxygen isotope ratios vary with latitude, altitude, and as you move inland from major bodies of water (Rubenstein & Hobson 2004).

Strontium and Lead

Strontium and lead isotope values reflect the soil makeup of a particular area, based on the age of the underlying bedrock. For instance, the isotope values for someone who grew up in Florida (younger limestone) would be very different from someone from northern Pennsylvania (older shale) (Beard & Johnson 2000).

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Q. *Why use teeth?*

A. Teeth are ideally suited for isotope studies because of their tough outer enamel layer. Enamel does not exchange materials with the surrounding environment once the tooth stops growing, so they are much less susceptible to the effects of contamination than bone. (Lee-Thorp & Sponheimer 2003) Formation of the enamel portion of permanent teeth continues until approximately 15 years of age (Hillson 1996), with each tooth varying based upon when it develops, but consistent between people of diverse backgrounds. These factors, therefore, allow teeth to provide a snapshot of the nutritional ecology of the childhood of a specific individual during the period of enamel formation.

Q. *How are military identifications currently performed?*

A. "Over the past 200 years, the United States has set the standard for the identification and return of its servicemembers to their families." (AFIP) Traditional measures include fingerprints, pilot footprints, DNA, medical and dental records, documented distinguishing marks such as scars and tattoos, and information such as hair and eye color and height and weight. Unfortunately, high impact crashes and the destructive forces of modern weaponry can, in certain instances, leave only a handful of remains behind, rendering most of these methods inadequate.

Q. *What are the limitations of DNA in identification efforts?*

A. Today, all members of the U.S. Armed Forces have bloodstain cards on file in the instance their nuclear DNA needs to be sequenced. This was not the case prior to the early 1990's (AFIP). Unless there are antemortem (prior to death) or familial profiles (maternally related relatives for mitochondrial DNA testing) to compare DNA against, such procedures are of limited use. Additionally, DNA is delicate and often cannot be extracted from severely damaged and/or heavily processed (and thus contaminated) bones and teeth or remains that have been exposed to the elements for a long period of time. This is well exemplified by the problems encountered in identifying those who died during the events of 11 Sep 2001. At the end of DNA processing, 2,749 victims were thought to have died at the World Trade Center site (Hirschkorn 2005). While a total of 19,916 human remains were recovered from the area in and around the Twin Towers, the physical destruction was so great that only 1585 individuals, or 58%, were identified. Over 9700 specimens remain unidentified (Hirschkorn 2005) either due to the inability to extract DNA from them or the absence of a reference sample to compare them against. Furthermore, compared to DNA processing, isotope analyses are quicker, less expensive, and much less vulnerable to contamination.

Q. *What good will come from this study?*

A. This study is groundbreaking in that it is the first of its kind to compile a reference sample of isotopic values associated with known childhood regions to be utilized in forensic work. More importantly, the information gathered will be applied in support of the JPAC's mission to achieve the fullest possible accounting of all Americans missing as a result of our nation's past conflicts. This process will prove especially useful in instances where DNA is unavailable or there is no reference sample to compare a profile against, particularly when the remains of many people are mixed together. Additionally, applications of this technology can extend to assisting in identification efforts in mass disasters such as airline crashes and the events of 11 September 2001 and in the identifications of illegal immigrants who die crossing our borders.

Q. *Were can I find additional information?*

A. We truly appreciate your consideration of participating in this worthy project. If you have any further questions, please do not hesitate to ask your dental staff or contact the associate investigator, Major Laura Regan at (352) 392-6772. Further sources of background information are listed on the following page.

Until they are home...

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APPENDIX B
CENTRAL IDENTIFICATION LABORATORY SAMPLING

Table B-1. Central Identification Laboratory sampling data.

Identifier	Sample	Origin	Tooth	Drill/ Loose	Chip Wt	Powder Wt	Comments
CIL-001	*01A*	Vietnam	16			0.0670	
	02A	Vietnam	32			0.1230	
CIL-002	*01A*	Vietnam	16			0.1057	
	02A	Vietnam	17			0.0892	
CIL-003	*01A*	Vietnam	17	D		0.0991	Carie bucco-medial cusp/chip same side
	02A	Vietnam	32	D		0.1181	Chipped while drilling
CIL-004	*01A*	Vietnam	1			0.0980	Very small
	02A	Vietnam	15			0.1137	
CIL-005	*01A*	Vietnam	1			0.1250	
	02A	Vietnam	32	D		0.1298	Partially impacted
CIL-006	*01A*	Vietnam	16			0.0730	Some wear
	02A	Vietnam	32			0.0891	Carie/mod calculus/ worn 2 dentine mesial 5mm wide
CIL-007	*01A*	Vietnam	17			0.1482	Mild calculus
	02A	Vietnam	32			0.1331	Moderate calculus
CIL-008	*01A*	Vietnam	32	D		0.0961	Sm side and center caries
	02A	Vietnam	31	D		0.0800	Minor calculus/caries center & lingual
CIL-009	*01A*	Solomon Isl.	18			0.1143	No 3rds/buccal carie
	02A	Solomon Isl.	31			0.1113	No 3rds/central & small side caries
CIL-010	*01A*	Vietnam	17			0.1158	Roots immature
	02A	Vietnam	32	D		0.1101	Roots immature/impacted/root abscess
CIL-011	*01A*	Vietnam	32	D	0.0120	0.1592	Rich red dirt/ 3 chips frm drilling in sep vial
	02A	Vietnam	31	D	0.0381	0.0774	Rich red dirt/mod wear/2 lrg chips frm drilling
CIL-012	*01A*	Vietnam	17			0.1632	Center carie

Table B-1. Continued.

Identifier	Sample	Origin	Tooth	Drill/ Loose	Chip Wt	Powder Wt	Comments
	02A	Vietnam	32			0.1427	
CIL-013	*01A*	Laos	~32	L		0.1262	Appears MNI=2 based on wear/articulation
CIL-014	*01A*	Vietnam	17	D	0.0687	0.1038	Chipped drilling out/3 chips
	02A	Vietnam	18			0.0723	Mild-mod wear
CIL-015	*01A*	Laos	17			0.1067	
	02A	Laos	32			0.1212	
CIL-016	*01A*	Vietnam	15		0.0307	0.0829	Glued in/nicked dentine but didn't use pwdr/3 chips
	02A	Vietnam	13	D		0.0550	Glued into alveoli
CIL-017	*01A*	Vietnam	~17	L		0.1896	Roots incomplete/ #1 impacted
	02A	Vietnam	~32	L		0.1700	
CIL-018	*01A*	Vietnam	31	D	0.0509	0.1700	
	02A	Vietnam	30		0.0097	0.0499	Heavy brown stain/req heavy cleaning
CIL-019	*01A*	Vietnam	1 (2)	L	0.0284	0.0508	Enamel chalky
	02A	Vietnam	16 (17)	L	0.1099	0.0777	Enamel chalky/roots encased red wax
CIL-020	*01A*	Vietnam	17 (18)	L		0.1970	Ambig place in jaw due to alveolar resorb/min wear
CIL-021	*01A*	Laos	1			0.1220	Minor wear/min chippng--did not keep
	02A	Laos	32			0.0927	Enamel chalky/heavy stain/cleaning
CIL-022	*01A*	Vietnam	18			0.1096	Rich red dirt/heavy stain/cleaning
CIL-023	*01A*	Vietnam	3	D		0.0843	Heavy wear/dentin exposed/heavy calculus
	02A	Vietnam	14	D	0.0338	0.0217	Heavy wear/dentin exposed/heavy calculus
CIL-024	*01A*	Vietnam	15			0.1043	Teeth black after cleaning/drilled stain off
	02A	Vietnam	14	D		0.0463	Same/chalky brown enamel undr black
CIL-025	*01A*	Vietnam	17			0.01485	

Table B-1. Continued.

Identifier	Sample	Origin	Tooth	Drill/ Loose	Chip Wt	Powder Wt	Comments
	02A	Vietnam	31	D		0.1782	
CIL-026	*01A*	Korea	18		0.0089	0.0362	No 3rds/heavy wear/occlusal surface dentin only
	02A	Korea	31			0.0503	Same but no chips/#6 bit
CIL-027	*01A*	Vietnam	19			0.0954	Buccal carie/3 small occlus caries
CIL-028	*01A*	Korea	2	D	0.2577	0.0489	Glued in/flaked apart on drilling
	02A	Korea	30		0.2431	-----------	Large chips prior to drilling
CIL-029	*01A*	Korea	17			0.0877	Center carie
	02A	Korea	18			0.0924	Center carie
CIL-030	*01A*	Vietnam	2	L	0.0121	0.0879	Mild wear
CIL-031	*01A*	Vietnam	18			0.0986	Crude black center fillng/mod wear
	02A	Vietnam	19	D	0.0686	0.0890	Mod wear/center & side caries
CIL-032	*01A*	Cambodia	18		0.0258	0.0681	Heavy brown stain
	02A	Cambodia	19	D	0.0733	0.0636	Glued in
CIL-033	*01A*	Cambodia	17		0.0323	0.0801	Brown stain
	02A	Cambodia	19		0.0649	0.0127	Heavy wear & chippng/little to work with
CIL-034	*01A*	Cambodia	18		0.0425	0.0568	No 3rds/heavy wear/thin wall left 2 work with
	02A	Cambodia	20			0.0526	No 3rds/heavy wear
CIL-035	*01A*	Vietnam	15	L		0.1164	No 3rds/2 center caries
	02A	Vietnam	31	L		0.1044	No 3rds/side carie
CIL-036	*01A*	Vietnam	19	L	0.0963	0.0357	Mod wear/black occlusal surf/mult chips/looks rough
CIL-037	*01A*	Cambodia	16			0.1392	Maybe dent mixd
	02A	Cambodia	15			0.1375	
CIL-038	*01A*	Vietnam	18		0.1038	0.0277	Heavy wear

Table B-1. Continued.

.Identifier	Sample	Origin	Tooth	Drill/ Loose	Chip Wt	Powder Wt	Comments
CIL-039	*01A*	Philippines	16		0.0408	0.1287	
	02A	Philippines	15			0.1166	Buccal carie
CIL-040	*01A*	Solomon Isl.	18			0.1897	
CIL-041	*01A*	Vietnam	32	D		0.1470	Occlusal surface brown
	02A	Vietnam	31	D		0.0987	Occlusal surface brown
CIL-042	*01A*	Vietnam	17			0.1164	Large center carie
	02A	Vietnam	31			0.1356	
CIL-043	*01A*	Vietnam	1			0.1750	Nasals more rounded
	02A	Vietnam	16			0.1895	4 roots
CIL-044	*01A*	Vietnam	32 (w)		0.0352	0.1069	Heavy cleanp/buccal & center caries/red mesial stain
	02A	Vietnam	31			0.0713	Very large lingual chips
CIL-045	*01A*	Vietnam	18 (w)		0.3215	0.0312	No 3rds/tooth fract upon drill/may be contaminated
	02A	Vietnam	31			0.1145	No 3rds/mult fracturess in other teeth
CIL-046	*01A*	Vietnam	17 (w)	D	0.0078	0.1232	Chip mes/ling cusp
	02A	Vietnam	31			0.1187	
CIL-047	*01A*	Vietnam	18 (w)			0.1403	
	02A	Vietnam	19	D	0.0615	0.1414	Glued & red wax/mild wear
CIL-048	*01A*	Vietnam	32 (w)			0.1246	Brown stain/smallish size
	02A	Vietnam	30		0.1210	0.0625	Light brown stain/heavy wear
CIL-049	*01A*	Vietnam	31 (w)			0.1333	
	02A	Vietnam	30			0.1225	Mod wear
CIL-050	*01A*	Vietnam	17 (w)			0.1714	Allignment of mandible 1/2s iffy

Table B-1. Continued.

Identifier	Sample	Origin	Tooth	Drill/ Loose	Chip Wt	Powder Wt	Comments
	02A	Vietnam	18			0.1630	
CIL-051	*01A*	Vietnam	32 (r)			0.0641	Distal surf gone/enamel flaked
	02A	Vietnam	30		0.0367	0.0664	Heavy wear
CIL-052	*01A*	Vietnam	30 (r)		0.0356	0.1241	No 3rd/lrg mesial, sm distal & lingual caries
CIL-053	*01A*	Vietnam	17(L)			0.0805	Large buccal chip
	02A	Vietnam	19			0.1348	Min wear
CIL-054	*01A*	Vietnam	17(L)			0.1344	Red wax/distal carie
	02A	Vietnam	19	D	0.0736	0.0703	Heavy wear
CIL-055	*01A*	Vietnam	18(17)			0.1451	Red wax
	02A	Vietnam	19(w)	L	0.0307	0.0747	Red wax/distal chip/heavy wear
CIL-056	*01A*	Vietnam	32(r)			0.1087	Red wax
	02A	Vietnam	31			0.1116	Mod-heavy wear/red wax
CIL-057	*01A*	Vietnam	31(w)	D	0.1852	0.1232	Large distal/lingual chip/red wax
	02A	Vietnam	19	D		0.0800	Heavy wear
CIL-058	*01A*	Vietnam	18(L)			0.1026	Large lingual chip/glued
	02A	Vietnam	19	D		0.1571	Glued/tough get out/heavy clean
CIL-059	*01A*	Vietnam	32(?)	D		0.1746	Allignment of mandible 1/2s iffy
	02A	Vietnam	31	D		0.1199	chose lrg chip over ? Tooth/#6
CIL-060	*01A*	Vietnam	17(w)			0.1679	Center/buccal carie
	02A	Vietnam	18			0.1218	
CIL-061	*01A*	Vietnam	32(w)	D		0.0789	1/2 crown missing

Joint POW/MIA Accounting Command
Central Identification Laboratory
Chain of Custody Form

Unique mission identifier N/A

CIL [redacted]

Evidence obtained from: JPAC-CIL 310 Worchester Ave. Bldg 45 Hickam AFB, HI 96853	**Evidence transferred to:** C.A. Pound Human Identification Laboratory, University of Florida Bldg 114, Radio Road Gainesville FL 32611-2545
Obtained by: Laura A. Regan, Maj, USAF	**Date obtained:** 17 Jun 05
Seal Number(s): N/A	**Village/District/Province or equivalent:** N/A
Associated Incident/Site: N/A	**Grid Coordinate:** N/A (Full MGRS and datum)
Conflict: SEA **Country:** Vietnam	

Item Number	Bag / Container Label and Description Provide Terminus Statement Following Last Entry, e.g., "Nothing Follows," Number all evidence bags / containers
1	Microcentrifuge tube labeled "CIL-011, #32" containing 0.1592 grams of enamel powder sampled from case CIL [redacted], tooth 32.
2	Microcentrifuge tube labeled "CIL-011, #32, chips" containing 0.0120 grams of enamel chips sampled from case CIL [redacted], tooth 32.
3	Microcentrifuge tube labeled "CIL-011, #31" containing 0.0774 grams of enamel powder sampled from case CIL [redacted], tooth 31.
4	Microcentrifuge tube labeled "CIL-011, #31, chips" containing 0.0381 grams of enamel chips sampled from case CIL [redacted], tooth 31.
---------	----------------------------Nothing Follows----------------------------

Item(s)	Transferred from:	Transferred to:	Date	Reason for Transfer
1-4	SIGNATURE PRINT NAME Laura A. Regan, Maj, USAF	SIGNATURE Tracking # 792359134148 PRINT NAME FEDERAL EXPRESS	16 Aug 05	Samples sent to C.A. Pound Lab for isotope analysis.
1-4	SIGNATURE Tracking # 792359134148 PRINT NAME FEDERAL EXPRESS	SIGNATURE PRINT NAME Laura A. Regan, Maj, USAF	22 Aug 05	Received for analysis
	SIGNATURE PRINT NAME	SIGNATURE PRINT NAME		
	SIGNATURE PRINT NAME	SIGNATURE PRINT NAME		

Number all pages – continue page sequence if second form is used.
JPAC FORM 007 APR 04

Page 1 of 1

Figure B-1. Sample CIL chain of custody form.

APPENDIX C
UNITED STATES AIR FORCE ACADEMY SURVEY RESULTS

USAFA Survey Code Key

Field	Code	Definition
Dates		day/month/year
Sex	1	Male
	2	Female
Tobacco Product Code	0	Non-user
	1	Inhalent
	2	Smokeless
	3	Both 1 & 2
Diet	1	Meat
	2	Vegetarian
	3	Vegan
	4	Changed regimes

Table C-1. United States Air Force Academy survey data.

				Tobacco Use					Residency				
Subject Identifier	**YOB**	**Sex**	**Race**	**Prod Code**	**Used From**	**Used Until**	**Frequency**	**Diet**	**From**	**To**	**City**	**State/ Country**	**Tooth #**
AFA-001	1984	1	Cauc	0	.	.	.	1	1984	1991	Coer D'Alene	ID	1
									1991	2002	Seaside	OR	16
									2002	2005	Colorado Springs	CO	32
AFA-002	1986	2	Cauc	0	.	.	.	1	1986	1996	Chicago	IL	1
									1996	1998	Iro	Peru	16
									1998	2003	Santa Ana	Costa Rica	17
									2003	2004	Leysin	Switzerland	32
AFA-003	1985	1	Hisp	0	.	.	.	1	1985	1997	Guaynabo	Puerto Rico	1
									1997	2000	Tampa	FL	16
									2000	2002	Newport News	VA	17
													32
AFA-004	1982	1	White	0	.	.	.	1	1982	1986	Clarksville	TN	1
									1986	1986	Cleveland	TN	16
									1986	2002	Clarksville	TN	17
									2002	2003	Marion	AL	32
									2003	2005	Clarksville	TN	
AFA-005	1986	2	White	0	.	.	.	1	1986	1988	West Islip	NY	13
									1988	2005	Syracuse	NY	16
AFA-006	1986	1	White	0	.	.	.	1	1986	2004	Laguna Niguel	CA	1
									2004	2005	Colorado Springs	CO	16
													32
AFA-007	1981	2	Black	0	.	.	.	1	1981	1991	Chicago	IL	1
									1991	1997	Colorado Springs	CO	16
									1997	1999	Las Vegas	NV	17
													32
AFA-008	1980	1	White	.	.	.	.	1	1980	1986	San Antonio	TX	16
									1986	2001	El Paso	TX	17
									2001	2005	Colorado Springs	CO	32
AFA-009	1984	2	Cauc/Asian	0	.	.	.	1	1984	2002	Greenville	SC	1
													16
AFA-010	1985	1	Cauc	3	2001	2005	1 pack/week	1	1985	1990	Camarillo	CA	1

Table C-1. Continued.

				Tobacco Use					Residency				
Subject Identifier	**YOB**	**Sex**	**Race**	**Prod Code**	**Used From**	**Used Until**	**Frequency**	**Diet**	**From**	**To**	**City**	**State/ Country**	**Tooth #**
					2002	2005	1 can/week		1990	1992	.	Guam	16
									1992	1998	Youngsville	NC	17
									1998	2003	Omaha	NE	32
AFA-011	1986	1	White	2	2000	2005	3-5 times/day	1	1986	1986	Wichita Falls	TX	1
									1986	1991	Davie	FL	16
									1992	2004	Manila	AR	17
									2004	2005	Colorado Springs	CO	32
AFA-012	1985	2	White	0	.	.	.	1	1985	1995	Hyde Park	VT	1
									1995	2001	Preston	ID	16
									2001	2003	Layton	VT	32
									2003	2004	Wichita Falls	TX	
									2005	2005	Colorado Springs	CO	
AFA-013	1985	1	Mex	0	.	.	.	1	1985	2003	Albuquerque	NM	1
													16
													17
													32
AFA-014	1984	1	Cauc	2	1995	2005	1 can/week	1	1984	2002	Georgetown	TX	1
									2002	2005	Colorado Springs	CO	32
AFA-015	.	1	Cauc	0	.	.	.	1	.	.	Omaha	NE	1
									.	.	Aurora	CO	16
									.	.	Colorado Springs	CO	17
													32
AFA-016	1985	1	Cauc	1	2002	2005	occasionally	1	1985	1986	Ft Walton Beach	FL	1
									1986	1988	Lutz	FL	16
									1988	1990	Virginia Beach	VA	17
									1990	1991	Birmingham	AL	32
									1991	1993	Marshall	TX	51
									1993	2001	Schell City	MO	66
									2001	2002	Ft Walton Beach	FL	
									2002	2005	Colorado Springs	CO	
AFA-017	1984	1	Hisp	1	.	.	1/day	1	1984	2002	Phoenix	AZ	1
									2002	2005	Colorado Springs	CO	16

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Used From	Used Until	Frequency	Diet	Residency: From	To	City	State/ Country	Tooth #
													32
AFA-018	1984	1	Cauc	0	.	.	.	1	1984	1993	Austin	TX	1
									1993	2002	Acworth	GA	16
AFA-019	1981	1	White	1	1998	2005	1/2 pack/day	1	1981	1983	Littleton	MA	17
									1983	1999	Everett	MA	
AFA-020	1984	2	White	0	.	.	.	1	1984	1985	Vail	CO	1
									1985	1992	Alexandria	VA	16
									1992	2002	San Antonio	TX	
									2002	2005	Colorado Springs	CO	
AFA-021	1983	1	White	2	2002	2005	1 can/week	1	1983	2002	Goodyear?	AZ	1
									2002	2005	Colorado Springs	CO	16
AFA-022	1984	2	Asian/White	0	.	.	.	1	1984	1995	Oceanside	CA	1
									1995	2000	Jacksonville	FL	16
													17
													32
AFA-023	1985	2	Pac Isl	0	.	.	.	1	1985	2003	Agat	Guam	1
									2003	2004	San Antonio	TX	16
									2004	2005	Colorado Springs	CO	17
													32
AFA-024	1985	2	.	3	2004	2004	3 pinches/day	1	1985	2003	Arvada	CO	1
					2003	2003	5/day		2003	2005	Colorado Springs	CO	16
													17
													32
AFA-025	1964	2	Cauc	0	.	.	.	1	1964	1988	Rochester	MI	17
AFA-026	1983	1	Cauc	0	.	.	.	1	1983	1984	Miami	FL	1
									1984	1989	Andrews AFB	MD	16
									1989	1994	Danville	PA	17
									1994	1995	Charlotte	NC	32
									1995	2002	San Antonio	TX	
									2002	2005	Colorado Springs	CO	
AFA-027	1982	1	White	0	.	.	.	1	.	.	.	UT	1
									.	.	.	AZ	16

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Used From	Used Until	Frequency	Diet	Residency: From	To	City	State/ Country	Tooth #
									.	.	.	UT	17
									.	.	.	CA	32
									.	.	.	UT	
										.	.	NM	
									.	.	.	TX	
									.	.	.	CO	
									.	.	.	Russia	
AFA-028	1982	1	Cauc	0	.	.	.	1	1982	2000	Katy	TX	1
													16
													17
													32
AFA-029	1985	1	White	0	.	.	.	1	1985	2003	Houston	TX	1
									2003	2005	Colorado Springs	CO	16
													17
													32
AFA-030	1984	1	Filipino	0	.	.	.	1	1984	1985	Oakland	CA	1
									1985	2002	Vallejo	CA	16
									2002	2005	Colorado Springs	CO	
AFA-031	1982	1	Hispanic	0	.	.	.	1	1982	2001	Denver	CO	1
									2001	2005	Colorado Springs	CO	16
													17
													32
AFA-032	1983	1	White	1	2004	2005	1 pack/week	1	1983	1997	Sprucegrove?	Alberta, Canada	1
									1997	2005	Roseburg	OR	16
													32
AFA-033	1984	1	White	0	.	.	.	1	1984	1999	Boston	MA	1
									1999	2002	Andover	MA	16
									2002	2005	Colorado Springs	CO	32
AFA-034	1984	1	White	0	.	.	.	1	1984	2003	Seattle	WA	1
									2003	2005	Colorado Springs	CO	16
													17
													32

Table C-1. Continued.

				Tobacco Use					Residency				
Subject Identifier	**YOB**	**Sex**	**Race**	**Prod Code**	**Used From**	**Used Until**	**Frequency**	**Diet**	**From**	**To**	**City**	**State/ Country**	**Tooth #**
AFA-035	1985	1	Asian	0	.	.	.	1	1985	1990	Flushing	NY	1
									1990	1993	.	NJ	16
									1993	1996	Flushing	NY	17
									1996	2004	Old Bridge	NJ	32
									2004	2005	Colorado Springs	CO	
AFA-036	1984	1	Cauc	0	.	.	.	1	1984	1985	Weymouth	MA	1
									1985	1990	Charlotte	NC	16
									1990	2002	Tryon	NC	17
													32
AFA-037	1983	1	Cauc	0	.	.	.	1	1983	1987	Nashville	TN	1
									1987	1990	Whitehouse	TN	16
									1990	2001	Portland	TN	
AFA-038	1985	1	Mexican	0	.	.	.	1	1985	1991	La Mirada	CA	1
									1991	1995	Hobbs	NM	17
									1995	1999	Alamogordo	NM	32
									1999	2003	El Paso	TX	
AFA-039	1982	1	White	1	2004	2005	twice a week	1	1982	1983	Salt Lake City	Utah	1
									1983	1985	Santa Maria	CA	16
									1985	1989	Abilene	TX	17
									1989	2002	Lubbock	TX	32
									2002	2005	Colorado Springs	CO	
AFA-040	1983	1	White	0	.	.	.	1	1983	1991	Greenville	SC	1
									1991	2002	Fort Mill	SC	32
AFA-041	1986	1	Afr Am	0	.	.	.	1	1986	1988	Phoenix	AZ	1
									1988	1994	UNK	Germany	16
									1994	1994	Fort Walton	FL	17
									1994	1997	Sheppard AFB	TX	32
									1997	2000	Alamogordo	NM	
									2000	2002	Keflivik	Iceland	
									2002	2005	Del Rio	TX	
									2005	2005	USAFA	CO	
AFA-042	1985	1	Afr Am	0	.	.	.	1	1985	1987	Barksdale AFB	LA	1

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
									1987	1989	Castle AFB	CA	17
									1989	1990	Winnipeg	Canada	32
									1990	1993	Plattsburg	NY	
									1993	1996	Pickerington	OH	
									1996	2003	Warner Robins	GA	
									2003	2005	Colorado Springs	CO	
AFA-043	1983	1	Black	0	.	.	.	1	1983	1989	Colorado Springs	CO	1
									1989	1996	Norcross	GA	16
									1996	2001	Anstell	GA	17
													32
AFA-044	1984	1	Cauc	0	.	.	.	1	1984	1989	Monte Vista	CO	1
									1989	1990	Lidge Field	MN	16
									1990	2003	Thief River Falls	MN	17
									2003	2005	USAF Academy	CO	32
AFA-045	1985	1	American	0	.	.	.	1	1985	1991	International Falls	MN	1
									1991	1995	Duluth	MN	16
									1995	1999	Carlsbad	NM	17
									1999	2003	Naples	FL	32
AFA-046	1986	1	Korean	0	.	.	.	1	1986	1990	Niles	IL	1
									1990	2004	Mundelein	IL	16
									2004	2005	Colorado Springs	CO	17
													32
AFA-047	1982	1	Asian	1	1999	1999	1/week	1	1982	1999	Taytay, Rizal	Phillipines	1
									1999	2003	UP Los Banos	Phillipines	5
									2003	2004	PMA Baynio City	Phillipines	12
									2004	2005	Colorado Springs	CO	16
													32
AFA-048	1986	1	White	0	.	.	.	1	1986	2004	New Braunfels	TX	1
									2004	2005	Colorado Springs	CO	16
													17
													32
AFA-049	1984	1	Cauc	0	.	.	.	1	1984	1986	Zanesville	OH	1

Table C-1. Continued.

				Tobacco Use					Residency				
Subject Identifier	**YOB**	**Sex**	**Race**	**Prod Code**	**Used From**	**Used Until**	**Frequency**	**Diet**	**From**	**To**	**City**	**State/ Country**	**Tooth #**
									1986	2003	Dover	OH	16
									2003	2005	Colorado Springs	CO	17
													32
AFA-050	1984	1	White	0	.	.	.	1	1984	1988	Spangdahlem AFB	Germany	17
									1988	1990	Las Vegas	NV	32
									1990	1994	Hahn AB	Germany	
									1994	1997	Tucson	AZ	
									1997	2003	Goldsboro	NC	
									2003	2005	Colorado Springs	CO	
AFA-051	1984	1	White	0	.	.	.	1	1984	2002	Arvada	CO	17
													32
AFA-052	1983	1	Filipino	0	.	.	.	1	1983	1987	Laspiñas, Manilla	Phillipines	1
									1987	2001	Anaheim	CA	16
									2001	2005	Colorado Springs	CO	17
AFA-053	1983	1	Cauc	2	2000	2005	2 cans/wk	1	1983	1998	Tampa	FL	1
									1998	2001	Faribault	MN	16
AFA-054	1986	1	Cauc	0	.	.	.	1	1986	1989	Enid	OK	1
									1989	1992	Rapid City	SD	16
									1992	1995	Colorado Springs	CO	17
									1995	1996	Rapid City	SD	32
									1996	1997	Monterey	CA	
									1997	2000	Buenos Aires	Argentina	
									2000	2001	West Springfield	WV	
									2001	2004	Athens	Greece	
									2004	2005	Colorado Springs	CO	
AFA-055	1985	1	Cauc	0	.	.	.	1	1985	1985	Oakland	CA	1
									1985	1988	Honolulu	HI	16
									1989	1992	NavCams	Guam	17
									1992	1994	Lexington Park	MD	32
									1994	2004	Sumner	WA	
									2004	2005	Colorado Springs	CO	
AFA-056	1985	1	White	0	.	.	.	1	1985	2003	Chaska	MN	17

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
													32
AFA-057	1985	1	White	0	.	.	.	1	1985	1988	San Mateo	CA	17
									1988	1995	Fremont	CA	32
									1995	2003	Tracy	CA	
AFA-058	1984	1	White	2	2002	2005	1 can/wk	1	1984	1985	State College	PA	1
									1985	2002	Centerville	VA	
									2002	2005	Colorado Springs	CO	
AFA-059	1985	1	White	0	.	.	.	1	1985	2003	West Milwaukee	WI	1
									2003	2005	Colorado Springs	CO	16
													17
													32
AFA-060	1985	1	Cauc	0	.	.	.	1	1985	2003	Kearney	NE	1
													16
													17
													32
AFA-061	1985	2	Other	0	.	.	.	1	1985	1987	San Mateo	CA	16
									1987	1989	Gathersburg	MD	17
									1989	2005	San Mateo	CA	32
AFA-062	1986	1	Cauc	1	2003	2005	2 pack/day	1	1986	1998	Allen	TX	16
									1998	2004	Bonham	TX	17
									2004	2005	Wichita Falls	TX	32
									2005	2005	St Louis	MO	
									2005	2005	Colorado Springs	CO	
AFA-063	1984	1	White	0	.	.	.	1	1984	1985	Riverside	CA	1
									1985	2002	Anchorage	AK	16
													17
													32
AFA-064	1982	1	Cauc	0	.	.	.	1	1982	1983	Boston	MA	1
									1983	1985	Albequerque	NM	16
									1985	1990	Dayton	OH	17
									1990	1993	Redlands	CA	32
									1993	2002	Dayton	OH	

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
									2002	2005	Colorado Springs	CO	
AFA-065	1984	2	White	0	.	.	.	1	1984	1988	Lakewood	CA	1
									1988	2002	Denver	CO	16
													17
													32
AFA-066	1985	1	Cauc	0	.	.	.	1	1985	1996	Bedwyn	IL	1
									1996	2003	Aurora	IL	16
									2003	2005	Colorado Springs	CO	17
													32
AFA-067	1983	1	Hispanic	1	.	.	1/week	1	1983	1987	NYC	NY	1
									1987	1988	Paris	France	16
									1988	1992	NYC	NY	17
									1992	1993	Hong Kong	China/UK	
									1993	2002	NYC	NY	
AFA-068	1984	1	Other	0	.	.	.	1	1984	1986	Scott AFB	IL	1
									1986	1987	???	MS	16
									1987	1989	Hickam AFB	HI	17
									1989	1992	Misawa AB	Japan	32
									1992	1996	Woodbridge	VA	
									1996	1998	Howard AB	Panama	
									1998	2005	Colorado Springs	CO	
AFA-069	1984	2	Cauc	0	.	.	.	4	1984	1987	Uppder Saddle River	NJ	1
									1987	1990	Ridgewood	NJ	16
									1990	1991	Paramus	NJ	17
									1991	2004	Allendale	NJ	32
									2004	2005	Paramus	NJ	
AFA-070	1984	1	Indian	0	.	.	.	1	1984	1991	Bronx	NY	1
									1991	2002	Coconut Creek	FL	16
									2002	2005	Colorado Springs	CO	
AFA-071	1985	1	Nat Hawaiian	2	2004	2005	3 per day	1	1985	1989	.	Japan	1
									1989	1992	San Pedro	CA	16
									1992	1996	San Antonio	TX	17

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Used From	Used Until	Frequency	Diet	Residency: From	To	City	State/ Country	Tooth #
									1996	2003	Bremerton	WA	32
									2003	2005	Colorado Springs	CO	
AFA-072	1985	1	Cauc/Korean	0	.	.	.	1	1985	1986	Fulda	Germany	1
									1986	1988	Radcliffe	KY	16
									1988	1992	??	TX	17
									1992	1994	??	MI	32
									1994	1998	Colonial Heights	VA	
									1998	2003	Midlothian	VA	
AFA-073	1985	2	Cauc	0	.	.	.	1	1985	2003	Athens	TX	1
									2003	2005	Colorado Springs	CO	16
													17
													32
AFA-074	1982	2	White	0	.	.	.	1	1982	1983	Valdosta	GA	16
									1983	1984	San Antonio	TX	17
									1984	2000	Cabot	AR	
AFA-075	1983	2	Cauc	0		.	.	.	1983	1993	Berlin	Germany	1
									1993	1995	Geilenkirchen	Germany	16
									1995	1997	Mons	Belgium	17
									1997	2002	Warner Robins AFB	GA	32
									2002	2005	Colorado Springs	CO	
AFA-076	1985	1	White	0	.	.	.	1	1985	1993	Redlands	CA	1
									1993	2004	Victorville	CA	
AFA-076B													16
													32
AFA-077	1984	1	White	0	.	.	.	1	1984	1994	Arlington	TX	1
									1994	2000	Burleson	TX	16
									2000	2002	Dover	NH	17
									2002	2003	Colorado Springs	CO	32
AFA-078	1985	1	White	0	.	.	.	1	1985	2003	Burlington	VT	1
									2003	2004	Kansas City	MO	16
									2004	2005	Colorado Springs	CO	17

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Used From	Used Until	Frequency	Diet	Residency: From	To	City	State/ Country	Tooth #
													32
AFA-079	1984	1	Cauc	0	.		.	1	1984	2000	Kalispell	MT	17
									2000	2002	Spokane	WA	
									2002	2003	Roswell	NM	
									2003	2005	Colorado Springs	CO	
AFA-080	1985	2	White	1	2000	2005	1/2 pack/day	1	1985	2003	Port Clinton	OH	1
									2003	2005	Colorado Springs	CO	16
													17
													32
AFA-081	1983	2	Cauc	0	.	.	.	1	1983	1985	Tumwater	WA	1
									1985	1987	Provo	UT	16
									1987	1991	Tri cities	WA	17
									1991	1994	Missoula	MT	32
									1994	2001	Lebanon	OR	
AFA-082	1987	1	Cauc	0	.	.	.	1	1987	2005	Boone	IA	1
													16
													17
													32
AFA-083	1983	2	Cauc	0	.	.	.	1	1983	2001	Jacksonville	TX	1
									2001	2001	Taichung	Taiwan	16
									2002	2005	Colorado Springs	CO	17
													32
AFA-084	1983	1	Hispanic	2	2004	2005	once a day	1	1983	1995	San Antonio	TX	1
									1995	1998	Irvine	CA	16
									1998	1998	Peoria	AZ	17
									1998	2002	San Antonio	TX	32
									2002	2005	Colorado Springs	CO	
AFA-085	1987	1	Cauc	0	.	.	.	1	1987	2005	Torrington	CT	1
									2005	2005	Colorado Springs	CO	16
													17
													32
AFA-086	1982	1	Cauc	0	.	.	.	1	1982	2001	Honolulu	HI	1

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
									2001	2005	Colorado Springs	CO	16
													17
													32
AFA-087	1984	2	White	0	.	.	.	1	1984	1985	Atlanta	GA	1
									1985	2003	Newnan	GA	16
													17
													32
AFA-088	1983	1	Cauc	1	2002	2005	1 every 2-3 mos	1	1983	1985	Wooster	OH	1
									1985	1986	South Bend	IN	16
									1986	2002	Wooster	OH	17
									2002	2005	Burbank	CA	32
AFA-089	1984	1	Cauc	2	2004	2005	3 x per wk	1	1984	1986	Columbus	OH	1
									1986	2003	Orlando	FL	16
									2003	2003	Crestline	CA	17
									2004	2005	Colorado Springs	CO	32
AFA-090	1985	2	White	0	.	.		1	1985	1988	Cary	NC	1
									1988	2003	Methuen	MA	16
									2003	2005	Colorado Springs	CO	17
													32
AFA-091	1985	1	White	0	.	.	.	1	1985	1990	Victoria	TX	1
									1990	1991	Albuquerque	NM	16
									1991	2004	Ft Davis	TX	17
									2004	2005	Colorado Springs	CO	32
AFA-092	1986	1	White	0	.	.	.	1	1986	1992	Jacksonville	NC	1
									1992	2004	Elon	NC	16
									2004	2005	Colorado Springs	CO	17
													32
AFA-093	1985	1	White	0	.	.	.	1	1985	2003	Cincinnati	OH	1
													6
													17
													32

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
AFA-094	1984	1	White	0	.	.	.	1	1984	2003	Summerville	SC	1
									2003	2005	Colorado Springs	CO	16
													17
													32
AFA-095	1973	1	Cauc	0	.	.	.	1	1973	1994	Scranton	PA	1
													16
													17
													32
AFA-096	1984	1	White	0	.	.	.	1	1984	2002	Bland	VA	1
													16
													17
													32
AFA-097	1986	1	Cauc	0	.		.	1	1986	2004	College Station	TX	1
									2004	2005	Colorado Springs	CO	16
													17
													32
AFA-098	1985	2	Cauc	3	2004	2005	each day	1	1985	1986	Honolulu	HI	1
					2005	2005	each day for 1 mo		1986	1988	Platte City	MO	5
									1988	2004	Corning	IA	12
									2004	2005	Colorado Springs	CO	16
													17
													20
													29
													32
AFA-099	1983	1	White	0	.	.	.	1	1983	1987	Evergreen	CO	1
									1987	2002	Littleton	CO	16
													32
AFA-100	1984	1	White	0		.	.	1	1984	1986	Valdosta	GA	1
									1986	1989	Apple Valley	CA	16
									1989	1992	Naples	Italy	17
									1992	1994	Ellensburg	WA	32
									1994	1995	Baker City	OR	

Table C-1. Continued.

				Tobacco Use					Residency				
Subject Identifier	**YOB**	**Sex**	**Race**	**Prod Code**	**Used From**	**Used Until**	**Frequency**	**Diet**	**From**	**To**	**City**	**State/ Country**	**Tooth #**
									1995	1996	Camdenton	MO	
									1996	1996	McNeal	AZ	
									1996	2000	Manila	Phillipines	
									2001	2002	Ellensburg	WA	
									2002	2003	Manila	Phillipines	
									2003	2005	Colorado Springs	CO	
AFA-101	1984	1	White	0	.	.	.	1	1984	1986	Springfield	MA	17
									1986	1989	Chicopee	MA	32
									1989	2002	Springfield	MA	
AFA-102	1985	1	White	2	2004	2005	1 can/mo	1	1985	2003	Sparta	NJ	1
													16
													17
													32
AFA-103	1983	1	Whte	0	.	.	.	1	1983	2002	Golden	CO	1
									2002	2005	Colorado Springs	CO	16
													17
													32
AFA-104	1983	1	White	0	.	.	.	1	1983	1986	Naples	Italy	1
									1986	1989	.	Maine	16
									1990	1998	Monrovia	MD	17
									1999	2001	Kailua	HI	32
									2001	2005	Colorado Springs	CO	
AFA-105	1983	1	White	0	.	.	.	1	1983	2001	Short Hills	NJ	1
													16
													17
													32
AFA-106	1984	1	Cauc	0	.	.	.	1	1984	1985	Norfolk	VA	1
									1985	1988	Charleston	SC	16
									1988	1992	Poway	CA	17
									1993	1993	Chesapeake	VA	32
									1994	2002	Longmeadow	MA	
									1996	1998	(summers only)	Guam	

Table C-1. Continued.

				Tobacco Use					Residency				
Subject Identifier	**YOB**	**Sex**	**Race**	**Prod Code**	**Used From**	**Used Until**	**Frequency**	**Diet**	**From**	**To**	**City**	**State/ Country**	**Tooth #**
									1998	2000	(same) Oceanside	CA	
									2000	2002	(same) Rocky Mount	NC	
AFA-107	1968	1	Wh	3	1984	1996	1/2 pack/day	1	1968	1971	Lebanon	OR	1
					1999	2005	1/2 can/day		1971	1986	Albany	OR	16
													17
													32
AFA-108	1987	1	Black	0	.	.	.	1	1987	1989	Watertown	NY	1
									1989	1993	Tacoma	WA	16
									1994	2000	Beaverton	OR	17
									2000	2005	Hillsboro	OR	
AFA-109	1986	2	Hispanic	0	.	.	.	1	1986	2004	Lima	Peru	1
									2004	2005	Colorado Springs	CO	16
													17
													32
AFA-110	1984	1	White	1	2002	2004	3-4 per yr	1	1984	1984	Newport News	VA	1
									1985	1987	Sumter	SC	16
									1987	2003	Southlake	TX	17
									2003	2005	Colorado Springs	CO	32
AFA-111	1984	1	white	0	.	.	.	1	1984	2002	Old Shasta	CA	1
													17
													32
AFA-112	1985	1	Cauc	0	.	.	.	1	1985	1986	McAllen	TX	1
									1986	1990	Tulsa	OK	16
									1990	2003	Albuquerque	NM	17
													32
AFA-113	1986	1	Black	0	.	.	.	1	1986	1998	Albany	GA	1
									1998	1999	Camilla	GA	16
									1999	2004	Albany	GA	17
									2004	2005	Leesburg	GA	32
AFA-114	1983	1	White	3	2004	2005	rarely	1	1983	1985	Ann Arbor	MI	1
					2004	2005	rarely		1985	1986	Los Angeles	CA	16
									1986	1993	Dallas	TX	32

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
									1993	2002	Atlanta	GA	
AFA-115	1985	1	White	0	.	.	.	1	1985	2003	Franklin	TN	1
													16
													17
													32
AFA-116	1975	1	Caucasian	2	1995	2005	1 can/week	1	1975	1994	Los Angeles	CA	1
													17
AFA-117	1985	2	Caucasian	0	.	.	.	1	1985	2004	Kent	WA	1
													16
													17
													32
AFA-118	1984	1	White	0	.	.	.	1	1984	1984	Loveland	CO	17
									1984	1989	Suanee	GA	32
									1989	1990	Lawrenceville	GA	
									1990	2002	Loganville	GA	
									2002	2005	USAFA	CO	
AFA-119	1979	1	White Cauc	1	1996	2005	1/2 pack	1	1979	1984	Barre	Vermont	1
									1984	1986	Greenfield	Mass	16
									1986	1999	Newport	Vermont	17
									1999	2002	Minot	North Dakota	32
									2002	2005	Colorado Springs	CO	
AFA-120	1980	2	African-Amer	0	.	.	.	1	1980	1988	Linden	Guyana	17
									1988	2004	Brooklyn	NY	32
									2004	2005	Colorado Springs	CO	
AFA-121	1986	1	White	0	.	.	.	1	1986	1991	Payson	UT	1
									1991	1993	Missoula	MT	16
									1993	2004	Florence	MT	17
AFA-122	1985	1	White	0	.	.	.	1	1985	1987	.	AL/LA	1
									1987	1990	Liverpool	NY	16
									1990	1996	North Syracuse	NY	17
									1996	2003	Havelock	NC	32
									2003	2005	Colorado Springs	CO	

Table C-1. Continued.

				Tobacco Use					Residency				
Subject Identifier	**YOB**	**Sex**	**Race**	**Prod Code**	**Used From**	**Used Until**	**Frequency**	**Diet**	**From**	**To**	**City**	**State/ Country**	**Tooth #**
AFA-123	1981	1	Cauc	0	.	.	.	1	1981	1982	Bryson City	NC	1
									1982	1984	??	AL	16
									1984	1986	Dothan	AL	17
									1986	1988	Greenville	MS	32
									1988	1991	Marysville	WA	
									1991	1994	Arlington	WA	
									1994	1995	Dothan	AL	
									1995	1998	Homestead	FL	
									1998	2000	Manheim	PA	
AFA-124	1984	1	White	1	2000	2005	1 pack per day	1	1984	1985	Greensboro	NC	1
									1985	1990	Trenton	MO	16
									1990	2003	Lincoln	NE	17
									2003	2004	.	TX	32
									2004	2005	Colorado Springs	CO	
AFA-125	1981	1	Caucasian	0	.	.	.	1	1981	2003	Oregon City	OR	1
													16
													17
													32
AFA-126	1985	1	Hispanic	0	.	.	.	1	1985	1997	Los Angeles	CA	1
									1997	2003	Benicia	CA	16
									2003	2005	Colorado Springs	CO	17
													32
AFA-127	1984	2	White	0	.	.	.	1	1984	1991	San Jose	CA	1
									1991	1996	Austin	TX	16
									1996	1997	San Jose	CA	17
									1997	2002	Pleasanton	CA	32
									2002	2005	Colorado Springs	CO	
AFA-128	1985	1	White	0	.	.	.	1	1985	1988	March AFB	CA	1
									1988	1991	Mather AFB	CA	16
									1991	1997	New Baden	IL	17
									1997	2003	Altus AFB	OK	32

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Used From	Used Until	Frequency	Diet	Residency: From	To	City	State/ Country	Tooth #
AFA-129	1986	1	White	1	2003	2005	1-3 per month	1	1986	1987	Charlotte	NC	1
									1987	1995	Coral Springs	FL	16
									1995	1996	West Palm Beach	FL	17
									1996	2004	Wantage	NJ	32
AFA-130	1982	2	Cauc	0	.	.	.	1	1982	1983	Ft Myers	FL	1
									1983	1987	Winchester	VA	16
									1987	1991	Cape Coral	FL	17
									1991	1993	Bealeton	VA	32
									1993	1994	Slanesville	WV	
									1995	2000	Inwood	WV	
AFA-131	1985	1	White	0	.	.	.	1	1985	2000	Esko	MN	1
									2000	2004	Clovis	CA	16
									2004	2005	USAF Academy	CO	17
													32
AFA-132	1984	1	White	0	.	.	.	1	1984	1986	Yuma	AZ	1
									1986	1989		AL	16
									1989	1992	Panama City	Panama	17
									1992	1995	Ramstein	Germany	32
									1995	2005	Colorado Springs	CO	
AFA-133	1986	2	White	0	.	.	.	1	1986	2004	Scottsboro	AL	1
									2004	2005	Colorado Springs	CO	16
													17
													32
AFA-134	1985	1	White	0	.	.	.	1	1985	2005	Southaven	MS	1
													16
													17
													32
AFA-135	1984	1	White	2	2003	2005	1can/week	.	1984	1988	Orlando	FL	1
									1988	1995	Grand Polanic	TX	16
									1995	1997	Louisville	KY	17
									1997	2003	Arlington	TX	32

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
									2003	2005	USAF Academy	CO	
AFA-136	1983	1	Caucasian	0	.	.	.	1	1983	2001	Jerome	ID	1
													16
													17
													32
AFA-137	1984	1	White	0	.	.	.	1	1984	1985	Gaithersburg	MD	1
									1985	1987	Shenandoah	VA	16
									1987	1992	Belfast	ME	17
									1992	2003	Newark	DE	32
									2003	2005	USAF Academy	CO	
AFA-138	1984	1	Asian	0	.	.	.	1	1984	2002	Gastonia	NC	1
													16
													17
													32
AFA-139	1982	2	Korean/Mexican	1	2003	2004	1pack/week	1	1982	1984	Colorado Springs	CO	1
									1984	1988	Los Angeles	CA	16
									1988	1991	Colorado Springs	CO	
									1991	1993	Panama City	Panama	
									1993	1996	Waniawa	HI	
									1996	1998	El Paso	TX	
									1998	1999	Vilseck	Germany	
									1999	2001	Heidelburg	Germany	
									2001	2005	Colorado Springs	CO	
AFA-140	1986	1	White	1	2003	2004	1/2 per month	1	1986	1988	Bitburg AB	Germany	1
									1988	1991	Luke AFB	AZ	16
									1991	1995	Eielson AFB	Alaska	17
									1995	1998	Hickam AFB	HI	32
									1998	2003	Lake Mary	FL	
AFA-141	1983	2	Asian	0	.	.	.	1	1983	1992	Seoul/Incheon	Korea	1
									1992	1995	West Point	NY	16
									1995	1998	Heidelburg	Germany	17

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
									1998	1999	London	England	32
									1999	2002	Seoul	Korea	
									2002	2005	Colorado Springs	CO	
AFA-142	1983	1	Caucasian	0	.	.	.	1	1983	1985	Austin	TX	1
									1985	1988	Goppingen	Germany	16
									1988	1991	Madrid	Spain	32
									1991	1994	Landstuhl	Germany	
									1994	2001	Enid	OK	
AFA-143	1983	1	Filipino-Amer	0	.	.	.	1	1983	1983	Ohahron	Saudi Arabia	1
									1983	1999	Seoul	Korea	16
													17
													32
AFA-144	1983	2	Mexican-Amer	0	.	.	.	1	1983	1984	San Diego	CA	1
									1984	1985	Yuma	AZ	16
									1985	1987	Landsdale	PA	17
									1987	1991	Cherry Point	NC	32
									1991	2000	Yuma	AZ	
									2000	2005	Colorado Springs	CO	
AFA-145	1986	1	Caucasian	0	.	.	.	1	1986	1986	Buffalo	WY	1
									1986	1991	Lake Charles	LA	
									1991	1993	Elida	OH	
									1993	1997	Cheyenne	WY	
									1997	1999	Glenrock	WY	
									1999	2004	Cheyenne	WY	
AFA-146	1983	1	White	0	.	.	.	1	1983	2001	Burley	ID	17
													32
AFA-147	1983	1	Caucasian	2	2001	2005	2/day	1	1983	1988	Oklahoma City	OK	1
									1988	2002	Tullahoma	TN	16
									2002	2005	USAF Academy	CO	17
AFA-148	1982	1	Asian	1	.	.	4 pieces/day	1	1982	2001	Yeosu	Korea	1
													16
													17

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
													32
AFA-149	1983	2	Caucasian	0	.	.	.	1	1983	2002	Chaffee	MO	1
									2002	2005	USAFA	CO	16
													17
													32
AFA-150	1985	1	White	0	.	.	.	1	1985	1987	Loring AFB	Maine	1
									1987	1988	Montgomery	Alabama	16
									1988	1991	Osan AFB/Soeul	Korea	17
									1991	1995	Grand Forks	ND	32
									1995	1999	Newport News	VA	
									1999	2002	Jakarta	Indonesia	
									2002	2004	Newport News	VA	
									2004	2005	USAF Academy	CO	
AFA-151	1983	1	Hispanic	0	.	.	.	1	1983	1985	Farmers Branch	TX	1
									1985	1986	Youngstown	OH	17
									1986	1988	Aromoore	OK	32
									1988	2002	Farmers Branch	TX	
									2002	2005	USAF Academy	CO	
AFA-152	1984	2	Asian	0	.	.	.	1	1984	1986	Yokota AFB	Japan	1
									1986	1989	Holoman AFB	NM	16
									1989	1993	Yokota AFB	Japan	
									1993	1998	Ramstein AFB	Germany	
									1998	2002	Brookings	SD	
									2002	2005	USAF Academy	CO	
AFA-153	1985	1	Whit	0	.	.	.	1	1985	2004	Pittsburg	PA	1
									2004	2005	USAF Academy	CO	16
													17
													32
AFA-154	.	1	Caucasian	0	.	.	.	1	.	.	Schaumburg	IL	1
									.	.	Peachtree City	GA	16
									.	.	USAF Academy	CO	17
													32

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
AFA-155	1981	1	White	0	.	.	.	1	1981	1985	Salt Lake City	Utah	1
									1985	1999	Allen	TX	16
									1999	2001	USAFA	CO	17
									2001	2003	Siberia	Russia	32
									2003	2005	USAFA	CO	
AFA-156	1985	1	White-Hispanic	0	.	.	.	1	1985	1987	Ft. Worth	TX	17
									1987	2004	Cleburne	TX	
AFA-157	1985	2	White/Non-Hispanic	0	.	.	.	1	1985	2003	Norfolk	NE	1
									2003	2005	Colorado Springs	CO	16
													17
													32
AFA-158	1987	1	White	0	.	.	.	1	1988	1990	Monterey	CA	1
									1990	2000	Columbia	MD	16
									2000	2005	Hanover	MD	17
													32
AFA-159	1986	2	White	0	.	.	.	1	1986	1988	San Diego	CA	1
									1988	1991	Monterrey	CA	16
									1991	1992	Newport	RI	17
									1992	1994	Goose Creek	SC	32
									1994	1997	Puyallup	WA	
									1997	2003	Germantown	MD	
									2003	2005	USAFA	CO	
AFA-160	1986	1	Caucasian	0	.	.	.	1	1986	1988	Ronkonkoma	NY	1
									1988	2004	Boca Raton	FL	16
									2004	2005	McKinney	TX	17
													32
AFA-161	1983	1	White	.	.	.	.	1	1983	1988	Cheboygan	MI	1
									1988	1989	Las Vegas	NV	16
									1989	2002	Cheboygan	MI	17
									2002	2002	Keesler AFB	MS	32
									2002	2004	Spangdahlem AB	Germany	

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
									2004	2005	Colorado Springs	CO	
AFA-162	1987	1	Korean/White	0	.	.	.	1	1987	.	Bethesda	MD	1
									.	.	Seattle	WA	16
									.	1993	Key West	FL	17
									1993	1996	Sault St. Marie	MI	32
									1996	1997	Waldorf	MD	
									1997	1999	Concord	CA	
									1999	2001	Alameda	CA	
									2001	2002	Waldorf	MD	
									2002	2005	Indian Head	MD	
									2005	2005	USAF Academy	CO	
AFA-163	1984	2	Hispanic	0	.	.	.	1	1984	2002	Houston	TX	1
									2002	2005	Colorado Springs	CO	16
AFA-164	1984	1	White	2	2004	2005	1 can/4 days	1	1984	2002	Washington	GA	1
									2002	2005	Colorado Springs	CO	16
													17
AFA-165	1983	1	White	0	.	.	.	1	1983	1993	Mt. Gilead	OH	1
									1993	1996	Kissimmee	FL	16
									1996	2002	Mt. Gilead	OH	17
													32
AFA-166	1984	1	Caucasian	1	2004	2005	1/4 pack per day	1	1984	2003	Bismarck	ND	1
													16
													17
													32
AFA-167	1984	1	White	0	.	.	.	1	1984	1987	Santa Fe	TX	1
									1987	2003	Alvin	TX	16
									2003	2005	USAFA	CO	17
													32
AFA-168	1984	1	Black	0	.	.	.	1	1984	2005	Monroeville	PA	1
													16
													17

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
													32
AFA-169	1983	1	White	0	.	.	.	1	1983	1998	Grundy Center	IA	1
									1998	2001	Dodge Center	MN	16
													17
													32
AFA-170	1985	1	Caucasian	0	.	.	.	1	1985	1990	Indinapolis	IN	1
									1990	1992	Madison	WI	16
									1992	2003	Watertown	WI	17
									2003	2005	Colorado Springs	CO	32
AFA-171	1985	1	African-Amer	0	.	.	.	1	1985	2003	Vauxhall	NJ	1
													16
													17
													32
AFA-172	1983	1	White	0	.	.	.	1	1983	2001	Houston	TX	1
									2001	2002	Lexington	MO	16
									2002	2005	USAF Academy	CO	17
													32
AFA-173	1984	2	Caucasian	0	.	.	.	1	1984	2002	Vacaville	CA	1
													16
AFA-174	1984	1	White	0	.	.	.	1	1984	2003	Elizabethtown	KY	1
									2003	2005	USAFA	CO	16
													17
													32
AFA-175	1986	1	White	1	2004	2005	2-3/week	1	1986	1995	Denver	CO	17
									1995	1997	Atlanta	GA	32
									1997	2000	Lake Oswego	OR	
									2000	2005	Leesburg	VA	
AFA-176	1984	1	White/Caucasian	0	.	.	.	1	1984	2003	Tallahassee	FL	1
									2003	2005	Colorado Springs	CO	16
													32
AFA-177	1984	1	.	0	.	.	.	1	1984	.	Ceiba	Puerto Rico	1
									.	1991	Peachtree City	GA	16

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
									1991	1994	Wiesbaden	Germany	
									1994	2003	Fairfax	VA	
									2003	2005	Colorado Springs	CO	
AFA-178	1984	1	Caucasian	0	.	.	.	1	1984	1985	Dyersburg	TN	16
									1985	1987	Dotham	AL	17
									1987	1994	Niceville	FL	
									1994	1997	Incirlik AB	Turkey	
									1997	2002	Fairborn	OH	
									2002	2003	Tallahassee	FL	
									2003	2005	Colorado Springs	CO	
AFA-179	1984	1	White	0	.	.	.	1	1984	1986	Lancaster	OH	1
									1986	2003	Columbus	OH	16
													17
													32
AFA-180	1985	2	White	0	.	.	.	1	1985	1992	Miami	FL	1
									1992	2002	Coral Springs	FL	16
									2002	2005	USAF Academy	CO	17
													32
AFA-181	1984	2	White/Hispanic	0	.	.	.	1	1984	2003	Houston	TX	1
													16
													17
													32
AFA-182	1984	1	Caucasian	0	.	.	.	1	1984	2002	East Stroudsburg	PA	1
									2002	2005	USAF Academy	CO	16
													17
													32
AFA-183	1980	2	White	0	.	.	.	1	1980	1996	La Quinta	CA	1
									1996	1997	Palm Desert	CA	3
									1997	2001	Ft. Carson	CO	16
									2001	2002	La Quinta	CA	17
									2002	2005	USAF Academy	CO	32
AFA-184	1982	2	Caucasian	0	.	.	.	1	1982	1983	Veliko Tarnovo	Bulgaria	1

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Used From	Used Until	Frequency	Diet	Residency: From	To	City	State/ Country	Tooth #
									1983	1995	Nova Zagora	Bulgaria	16
									1995	1997	Tripoli	Libya	17
									1997	2000	Nova Zagora	Bulgaria	32
AFA-185	1984	1	White	0	.	.	.	1	1984	2002	Corcoran	CA	16
									2002	2006	USAFA	CO	17
AFA-186	1984	1	Caucasian	0	.	.	.	1	1984	2002	Inola	OK	1
													16
													17
													32
AFA-187	1983	1	Caucasian	3	2002	2006	1/week	1	1983	2002	Indianapolis	IN	1
					2005	2006	2/week		2002	2006	Colorado Springs	CO	16
													17
													32
AFA-188	1983	1	Caucasian	0	.	.	.	1	1983	1989	Grand Rapids	MI	1
									1990	2002	Spring Lake	MI	16
									2002	2005	USAFA	CO	32
AFA-189	1986	1	Caucasian	2	2003	2006	1/week	1	1986	1995	Belmont	CA	17
									1995	1997	High Springs	FL	32
									1997	2004	Sandy	UT	
									2004	2006	USAFA	CO	
AFA-190	1985	1	White	0	.	.	.	1	1985	1990	Kansas City	KA	1
									1990	1996	North Bend	WA	16
									1996	2003	Mercer Island	WA	17
													32
AFA-191	1981	1	Caucasian	0	.	.	.	1	1981	1986	Syracuse	NY	1
									1986	1994	Houghton	LA	16
									1994	2000	Manhawkin	NJ	17
									2000	2001	Dixon	CA	32
									2001	2005	USAFA	CO	
AFA-192	1984	1	Caucasian	0	.	.	.	1	1984	2002	Portland	OR	16
													17
AFA-193	1984	1	White	0	.	.	.	1	1984	1990	Fresno	CA	16

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Used From	Used Until	Frequency	Diet	Residency: From	To	City	State/ Country	Tooth #
									1990	2002	Modesto	CA	
AFA-194	1983	1	Hispanic	3	1999	Present	2/week	1	1983	1984	Ft. Stuart	GA	1
					1999	Present	2/week		1984	2006	Colorado Springs	CO	16
AFA-195	1984	1	Caucasian	0	.	.	.	1	1984	2002	Hackettstown	NJ	1
									2002	2005	USAFA	CO	16
AFA-196	1980	1	W	2	1999	2004	daily	1	1980	1999	Bonaire	GA	17
AFA-197	1983	1	Caucasian	0	.	.	.	1	1983	2002	St. Johns	MI	1
													16
AFA-198	1983	1	Caucasian	0	.	.	.	1	1983	2001	Brooklyn Park	MN	1
													16
													17
													32
AFA-199	1983	1	Caucasian	0	.	.	.	1	1983	2004	Dallas	TX	1
									2004	2006	USAFA	CO	17
AFA-200	1983	1	Caucasian	0	.	.	.	1	1983	1984	Houston	TX	1
									1984	2002	Carlbad	NM	16
													17
													32
AFA-201	1983	1	Caucasian	0	.	.	.	1	1983	2002	New Castle	IN	1
									2002	2005	USAFA	CO	16
													17
													32
AFA-202	1984	1	Asian	0	.	.	.	1	1984	1996	Chicago	IL	16
									1996	2002	Mundelein	IL	
AFA-203	1984	2	White	0	.	.	.	1	1984	2003	Burnsville	MN	1
													16
													17
													32
AFA-204	1983	1	Caucasian	0	.	.	.	1	1983	2002	Buffalo Grove	IL	1
													16
AFA-205	1983	1	Caucasian	2	2002	2005	4/week	1	1983	2002	Atlanta	GA	1
									2002	2006	Colorado Springs	CO	16

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
													17
													32
AFA-206	1983	1	White	0	.	.	.	1	1983	1992	Portland	TX	1
									1992	1997	Eagan	MN	16
									1998	2001	Portland	TX	32
									2001	2006	USAFA	CO	
AFA-207													
AFA-208	1984	1	Caucasian	0	.	.	.	1	1984	1989	Arvada	CO	1
									1989	2002	Broomfield	CO	16
									2002	2006	USAFA	CO	17
													32
AFA-209	1985	1	Caucasian	0	.	.	.	1	1985	1986	Norfolk	VA	16
									1986	1989	LasVegas	NV	17
									1989	1992	Bitburg	Germany	
									1992	1993	Clifton	VA	
									1993	2006	Colorado Springs	CO	
AFA-210	1984	1	White	0	.	.	.	1	1984	1984	Red Bank	NJ	1
									1984	1986	Ft. Leavenworth	KS	16
									1986	1989	Ft. Bliss	TX	17
									1989	1992	Kitzogen	Germany	32
									1992	1997	Ft. Hood	TX	
									1997	2000	Ft. Riley	KS	
									2000	2002	Ft. Hood	TX	
AFA-211	1983	1	Caucasian	0	.	.	.	1	1983	1986	Houston	TX	1
									1986	2002	Park City	UT	17
									2002	2006	Colorado Springs	CO	32
AFA-212	1983	2	White	0	.	.	.	1	1983	2002	Butte	MT	1
													16
													17
													32
AFA-213	1984	1	Caucasian	0	.	.	.	1	1984	1985	Ft. Stewart	GA	1
									1985	1988	Ft. Lee	VA	16

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Used From	Used Until	Frequency	Diet	Residency: From	To	City	State/ Country	Tooth #
									1988	1990	Ft. Leavenworth	KS	32
									1990	1991	Heidelberg	Germany	
									1991	1993	Reichenbach	Germany	
									1993	1997	Carbondale	IL	
									1997	2002	Davidson	NC	
									2002	2003	Colorado Springs	CO	
AFA-214	1986	1	Pacific Island	0	.	.	.	1	1986	1994	Vallejo	CA	1
									1994	2000	Puna	HI	16
									2000	2004	Kapolei	HI	17
													32
AFA-215	1984	2	Multiracial	0	.	.	.	1	1984	1993	Rock Island	IL	1
									1993	2002	Moline	IL	16
									2002	2003	Cedar Rapids	IA	
									2002	2003	Urbana	IA	
AFA-216	1985	2	White	0	.	.	.	1	1985	1986	West Point	NY	17
									1986	1989	Alamagordo	NM	32
									1989	1990	Brighton	MI	
									1990	1995	Fairfax	VA	
									1995	2005	Manassas	VA	
AFA-217	1984	1	White	0	.	.	.	1	1984	1986	Munich	Germany	1
									1986	2002	Knoxville	TN	16
									2002	2006	Colorado Springs	CO	17
													32
AFA-218	1984	1	White	0	.	.	.	1	1984	2002	Hicksville	NY	1
													16
													17
													32
AFA-219	1984	1	White	0	.	.	.	1	1984	2003	Bay City	TX	16
									2003	2006	USAFA	CO	32
AFA-220	1983	1	Mixed	0	.	.	.	1	1983	2004	Paramaribo	Suriname	1
													5
													12

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
													16
													17
													32
AFA-221	1984	1	White	1	2005	2005	2/week	1	1984	2003	Nitro	WV	1
									2003	2006	USAFA	CO	16
													17
													32
AFA-222	1986	1	White	0	.	.	.	1	1986	2004	Tulsa	OK	1
									2004	2006	USAFA	CO	16
AFA-223	1984	2	White	0	.	.	.	1	1984	2003	Laguna Niguel	CA	1
													16
													17
													32
AFA-224	1982	1	Caucasian	0	.	.	.	1	1982	2000	Boulder	CO	1
									2000	2001	Roswell	NM	16
									2001	2006	Colorado Springs	CO	17
													32
AFA-225	1983	1	Caucasian	2	2002	2005	not often	1	1983	1986	Lansing	MI	1
									1986	2001	Phoenix	AZ	17
AFA-226	1983	1	White	0	.	.	.	1	1983	1989	Vancouver	WA	1
									1989	2001	Billings	MT	16
													17
													32
AFA-227	1984	1	White	0	.	.	.	1	1984	1984	Knoxville	TN	1
									1984	1990	Lawrenceville	GA	16
									1990	2002	Spartanburg	SC	17
													32
AFA-228	1984	1	Caucasian	0	.	.	.	1	1984	2003	Wheeling	WV	1
													16
													17
													32
AFA-229	1985	1	Caucasian	1	2004	2005	1pk/month	1	1986	1986	Oakland	CA	1

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
									1986	1988	Memphis	TN	16
									1988	1990	???	WA	17
									1990	1993	Lubbock	TX	32
									1993	1995	Corpus Christi	TX	
									1995	1996	Pensacola	FL	
									1996	1999	Crofton	MD	
									1999	2002	.	.	
AFA-230	1985	1	White	0	.	.	.	1	1985	2000	Montgomery	IL	1
									2000	2003	Yorkville	IL	16
													17
													32
AFA-231	1984	1	White	0	.	.	.	1	1984	2002	Grand Haven	MI	1
									2002	2006	Colorado Springs	CO	16
													17
													32
AFA-232	1985	1	White	0	.	.	.	1	1985	1987	Ventura	CA	1
									1987	1990	Long Beach	CA	16
									1990	1998	Tehachupi	CA	17
									1998	2003	Mission Viejo	CA	32
									2003	2005	USAFA	CO	
AFA-233	1984	1	Caucasian	0	.	.	.	1	1984	1992	Limestone	ME	17
									1992	1998	Plattsburgh	NY	32
									1998	2002	Niagra Falls	NY	
AFA-234	1984	2	Caucasian	0	.	.	.	1	1984	1985	Enid	OK	1
									1985	1986	Washington DC		16
									1987	1991	Plattsburgh	NY	17
									1991	1994	San Antonio	TX	32
									1994	1997	Lubbock	TX	
									1997	2003	San Antonio	TX	
AFA-235	1986	2	White	0	.	.	.	1	1986	2004	Cincinnati	OH	1
									2004	2006	Colorado Springs	CO	16
													17

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
													21
													28
													32
AFA-236	1983	1	Caucasian	0	.	.	.	1	1983	1984	Del Rio	TX	1
									1984	1986	Arlington	VA	16
									1986	1987	Plattsburgh	NY	
									1987	1991	York	ME	
									1991	1994	Vienna	VA	
									1994	1998	Dallas	TX	
									1998	2002	Virginia Beach	VA	
									2002	2006	Colorado Springs	CO	
AFA-237	1985	2	Caucasian	0	.	.	.	1	1985	1990	Colome	SD	17
									1990	1992	Utica	NE	32
									1992	1995	Colome	SD	
									1995	2004	Gregory	SD	
AFA-238	1983	1	Caucasian	0	.	.	.	1	1983	1989	Eglin AFB	FL	1
									1989	1992	Missawa AFB	Japan	16
									1992	1998	Holloman AFB	AZ	17
									1998	2000	Tucson	AZ	32
									2000	2001	Hickam AFB	HI	
									2001	2006	USAFA	CO	
AFA-239	1985	1	White	0	.	.	.	1	1985	1988	White Haven	PA	16
									1988	1989	Natchez	MI	17
									1989	1991	Durham	NC	32
									1991	1998	Cape Cod	MA	
									1998	2002	Corpus Christi	TX	
									2002	2005	Colorado Springs	CO	
AFA-240	1976	1	Caucasian	0	.	.	.	1	1976	1990	Denton	TX	1
									1990	1995	Pueblo	CO	16
									1995	2003	Denton	TX	17
													32
AFA-241	1984	1	White	0	.	.	.	1	1984	1985	Wright-Pat AFB	OH	1

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
									1985	1988	Colorado Springs	CO	16
									1988	1990	Chanute AFB	IL	17
									1990	2002	Colorado Springs	CO	32
AFA-242	1983	1	Caucasian	0	.	.	.	1	1983	1985	Corona del Mar	CA	1
									1985	2002	St Helena	CA	16
									2002	2006	Colorado Springs	CO	17
													32
AFA-243	1983	1	Caucasian	0	.	.	.	1	1983	1985	Hope	MI	1
									1985	1986	Grand Blanc	MI	16
									1986	1990	Hope	MI	17
									1990	1993	Franklin	TN	32
									1993	2002	Murfreesboro	TN	
									2002	2006	USAFA	CO	
AFA-244	1984	1	White	0	.	.	.	1	1984	1989	Salinas	CA	1
									1989	2003	King City	CA	16
													17
													32
AFA-245	1985	1	Hispanic	0	.	.	.	1	1985	1985	El Centro	CA	1
									1985	1992	Kearny	AZ	6
									1992	2002	Albuquerque	NM	16
									2002	2003	Carlsbad	NM	17
													32
AFA-246	1984	2	White	0	.	.	.	1	1984	2003	Kalispell	MT	1
									2003	2006	USAFA	CO	16
													17
													32
AFA-247	1984	1	White	0	.	.	.	1	1984	1984	Port Chester	NY	1
									1984	1990	Yuba City	CA	16
									1990	1995	Oakland	OR	17
									1995	2002	Roseburg	OR	32
									2002	2006	Colorado Springs	CO	
AFA-248	1982	1	Caucasian	1	2001	2006	5 cigs/week	1	1982	1985	.	CA	1

Table C-1. Continued.

				Tobacco Use					Residency				
Subject Identifier	**YOB**	**Sex**	**Race**	**Prod Code**	**Used From**	**Used Until**	**Frequency**	**Diet**	**From**	**To**	**City**	**State/ Country**	**Tooth #**
									1985	1987	.	ND	16
									1987	1988	.	MI	17
									1988	1990	.	AZ	32
									1990	2001	.	CO	
									2001	2002	.	AL	
									2002	2006	.	CO	
AFA-249	1983	1	Caucasian	0	.	.	.	1	1983	1984	Fayetteville	NC	1
									1984	1985	Fort Jachuka	AZ	16
									1985	1988	Lenoir	NC	17
									1988	1994	Steamboat Springs	CO	32
									1994	1997	Wytheville	VA	
									1997	2002	Marion	VA	
AFA-250	1984	1	White	0	.	.	.	1	1984	2002	Los Angeles	CA	1
													16
													17
													32
AFA-251	1984	2	Caucasian	0	.	.	.	1	1984	1985	Lewisville	TX	1
									1985	1990	Morganton	NC	16
									1990	2002	Hoover	AL	17
									2002	2002	USAFA	CO	32
AFA-252	1986	1	Caucasian	0	.	.	.	1	1986	1987	Monteray	CA	1
									1987	1990	Woods Bridge	VA	16
									1990	2004	Orem	UT	17
									2001	2005	USAFA	CO	32
AFA-253	1984	1	Caucasian	0	.	.	.	1	1984	1985	Williams AFB	AZ	1
									1985	1987	McChord AFB	WA	16
									1987	1988	Cannon AFB	NM	32
									1988	1995	Lakenheath AB	England	
									1995	1999	Seymour Johnston AFB	NC	
AFA-254	1984	1	Asian	0	.	.	.	1	1984	1988	Durham	NC	1
									1988	1991	State College	PA	16
									1991	2002	Fredericksburg	VA	17

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
													32
AFA-255	1983	1	Caucasian	1	2003	2006	2/year	1	1983	1984	Stuttgart	Germany	1
									1984	1996	Manhattan	KS	17
									1996	1998	Seward	NE	
									1998	2002	Independence	KS	
AFA-256	1983	1	Black	0	.	.	.	1	1983	1984	Holloman AFB	NM	1
									1984	1991	McGuire AFB	NJ	16
									1991	1995	Eielson AFB	AK	17
									1995	2002	Baltimore	MD	32
AFA-257	1984	1	Caucasian	0	.	.	.	1	1984	2002	Montoursville	PA	1
									2002	2006	Colorado Springs	CO	16
													17
													32
AFA-258	1983	1	White	0	.	.	.	1	1983	1987	George AFB	CA	1
									1987	1992	Homestead AFB	FL	16
									1992	1993	Gunter AFB	AL	17
									1993	1995	Langley AFB	VA	32
									1995	1999	Mountain Home AFB	ID	
									1999	2000	Paris	France	
									2000	2002	Naples	Italy	
									2002	2006	USAFA	CO	
AFA-259	1983	2	Hispanic	0	.	.	.	1	1983	2002	San Diego	CA	1
									2002	2006	Colorado Springs	CO	16
													17
													32
AFA-260	1982	1	Caucasian	0	.	.	.	1	1982	1984	Drexell Hill	PA	1
									1984	1987	Satellite Beach	FL	16
									1987	1991	Greenville	PA	17
									1991	2001	King of Prussia	PA	32
									2001	2006	USAFA	CO	
AFA-261	1984	1	White	0	.	.	.	1	1984	1987	San Diego	CA	1
									1987	1990	Suore Point	Phillipines	16

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
									1990	1995	Meridian	MS	17
									1995	2002	Memphis	TN	32
									2002	2006	Colorado Springs	CO	
AFA-262	1986	1	Caucasian	0	.	.	.	1	1986	1986	Colorado Springs	CO	1
									1986	1999	Grand Rapids	MI	16
									1999	2003	Metamora	IL	17
									2003	2006	Colorado Springs	CO	32
AFA-263	1984	2	Indian	0	.	.	.	2	1984	1992	Nairobi	Kenya	17
									1992	2002	Old Bridge	NJ	32
									2002	2006	Colorado Springs	CO	
AFA-264	1984	1	White	0	.	.	.	1	1984	1990	Cincinnati	OH	1
									1990	2006	Barron	WI	16
													32
AFA-265	1979	2	White	0	.	.	.	1	1980	1998	Colorado Springs	CO	1
													16
													17
													32
AFA-266	1984	2	White	0	.	.	.	1	1984	1985	Harrogate	England	17
									1986	1988	Sacramento	CA	32
									1988	2001	Cameron Park	CA	
AFA-267	1982	2	White	0	.	.		1	1982	1983	Charleston	SC	1
									1983	1990	Monrovia	MD	16
									1990	2000	.	.	32
AFA-268	1984	1	White	0	.	.	.	1	1984	1984	Dayton	OH	1
									1985	1987	Ithaca	NY	16
									1987	1990	Omaha	NE	17
									1990	1993	Deddington	United Kingdom	32
									1993	1995	San Antonio	TX	
									1995	1999	Yokota	Japan	
									2000	2002	Dayton	OH	
AFA-269													
AFA-270	1984	1	White	0	.	.	.	1	1984	1985	Aurora	CO	1

Table C-1. Continued.

Subject Identifier	YOB	Sex	Race	Tobacco Use: Prod Code	Tobacco Use: Used From	Tobacco Use: Used Until	Tobacco Use: Frequency	Diet	Residency: From	Residency: To	Residency: City	Residency: State/ Country	Tooth #
									1985	2005	Colorado Springs	CO	16
AFA-271	1984	1	Anglo Sax/White	0	.	.	.	1	1984	1985	Goldsboro	NC	1
									1985	1988	George AFB	CA	32
									1988	1988	Sevel	Germany	
									1988	1990	Flavione	Belgium	
									1990	1992	Spangdelhm AFB	Germany	
									1992	1996	Smithfield	VA	
									1996	1999	Niborg	Denmark	
									1999	2000	Montgomery	AL	
									2000	2002	Belton	TX	
AFA-272	1982	1	Black	1	2001	2006	2 packs/year	1	1982	2001	Macon	GA	1
									2001	2006	USAFA	CO	16
													17
													32
AFA-273	1984	1	Caucasian	0	.	.	.	1	1984	2002	Easton	MD	1
													16
AFA-274	1983	1	White	0	.	.	.	1	1983	1987	Elmhurst	IL	1
									1987	2002	Inverness	FL	16
									2002	2006	Colorado Springs	CO	17
													32
AFA-275	1984	1	African American	0	.	.	.	1	.	.	.	VA	1
									.	.	New Brunswick	NJ	16
									.	.		NJ	17
									.	.	Brooklyn	NY	32
									.	.	Hamilton (Trenton)	NJ	
AFA-276	1985	1	White	0	.	.	.	1	1985	2003	Rochester	NY	1
													16
													17
													32

APPENDIX D
EXAMPLE PRISM LOAD SHEET

Isocarb Carbonate run sheet (Prism)

Who Loaded L Regan
Tray Number 3
Date Loaded 28 Apr 06

Who Analyzed
Date Analyzed

Line	Sample	Weight (mg)	Species	$\delta^{18}O$	$\delta^{13}C$	Comments
1	NBS-19	0.120	Std			
2	NBS-19	0.104	Std			
3	NBS-19	0.076	Std			
4	NBS-19	0.092	Std			
5	CIL-029 #18	1.044				
6	AFA-194 #1	1.192				
7	AFA-223 #32	1.014				
8	AFA-230 #17	1.094				
9	AFA-231 #32	1.088				
10	AFA-235 #17	1.210				
11	AFA-237 #32	1.150				
12	AFA-240 #32	1.218				
13	AFA-241 #1	1.078				
14	AFA-246 #16	1.192				
15	AFA-250 #32	1.066				
16	AFA-251 #1	1.158				
17	AFA-252 #16	1.118				
18	AFA-254 #1	1.088				
19	AFA-257 #16	1.064				
20	AFA-263 #17	1.008				
21	NBS-19	0.066	Std			
22	NBS-19	0.120	Std			
23	AFA-264 #1	1.140				
24	AFA-265 #16	1.000				
25	AFA-267 #32	1.058				
26	AFA-270 #16	1.118				
27	AFA-272 #16	1.186				
28	AFA-273 #1	1.158				
29	AFA-274 #17	1.112				
30	AFA-276 #32	1.002				
31	2CIL-010 #17	1.156				
32	3CIL-010 #17	1.092				
33	4CIL-010 #17	1.086				
34	5CIL-010 #17	1.204				
35	6CIL-010 #17	1.198				
36	2 CIL-045 #31	1.138				
37	3 CIL-045 #31	1.166				
38	4 CIL-045 #31	1.104				
39	5 CIL-045 #31	1.048				
40	6 CIL-045 #31	1.204				
41	2 AFA-038 #1	1.070				
42	3 AFA-038 #1	1.086				
43	NBS-19	0.096	Std			
44	NBS-19	0.122	Std			

450-500 Ideal
350-450 OK
(180-350) poor readings
80-180 OK

UF Geological Sciences Stable Isotope lab

Figure D-1. Example PRISM load sheet

APPENDIX E

COLUMN CHEMISTRY VESSEL AND IMPLEMENT CLEANING INSTRUCTIONS

DISHWASHING

Everyone who uses the beakers and vials in the clean lab is expected to help clean them.

1. Remove labels. If some glue or residue is left behind, use acetone to remove it.
2. Rinse out the beakers in house DI water (from the grey tap). Use a gloved finger to wipe the inside of the beaker and remove any stuck-on material.
3. Put the beakers in a soap and water bath. There are large containers under the sink for this purpose. The soap and water solution is made of house DI water and <u>few drops</u> of Versaclean soap.
4. Soak at least overnight in the soap and water.
5. NON-TEFLON: Remove from the soap and water bath, rinse with 2X H_2O and let dry on a Kimwipe by the sink.
6. TEFLON: Drain the soap and water solution from the container. Add house DI water, shake and rain it off. Repeat the rinse step until soap is gone.
7. Put the beakers in the 50% (7M) HNO_3 bath. (Note: the HNO_3 bath should always be left in the fume hood!)
8. Let sit overnight or longer.
9. Add a little 2X H_2O to clean Tupperware container.
10. Remove beakers from the bath using tongs. Place them in the clean Tupperware.
11. Add enough 2X H_2O to cover the beakers, swish it around, and dump it out.
12. Repeat Step 11 two more times
13. Put the beakers in the 50% HCl bath (This bath contains half concentrated trace metal grade HCl and half 4X H_2O).
14. Add a little 2X H_2O to the clean Tupperware container.
15. Remove beakers from the bath using tongs. Place them in the clean Tupperware.
16. Add enough 2X H_2O to cover the beakers, swish it around, and dump it out.
17. Repeat Step 16 two more times.
18. Put the beakers in the 2X H_2O bath.
19. Let sit overnight or longer.
20. Drain off the H_2O
21. Fill the container with 2X H_2O, swish it around and dump it out.
22. Repeat Step 21 twice.
23. Put the beaker and caps in a laminar flow hood, on a lint-free wiper. Cover loosely with parafilm and allow to dry.
24. Do not let the beakers sit for days and days “drying”, as they will pick up dust.
25. When they are dry, put the caps on and put them away in the drawer in Bay 2.

PIPETTE TIPS

1. Place purchased pipette tips, tip down in a Teflon cup.
2. Pre-leach Sr & Pb from the plastic pipette tips by covering the tips, inside and out, with 6N HCl.
3. Shake out any air bubbles and fill to below the cup rim.
4. Place the covered cup in the fume hood overnight, on a labeled napkin.
5. Rinse tips twice in 4X H_2O and leave soaking overnight in a third wash of 4X H_2O.
6. Rinse tips twice in 4X H_2O and shake out any excess water.
7. Put the cup of tips on the top shelf of the laminar flow hood to dry. Remove the lid, and place it askew on the cup leaving as much of the tips exposed to the passing air as you can.
8. Once dry, recap the cup and place it in the drawer of clean pipette tips.

APPENDIX F
MISCELLANEOUS HEAVY ISOTOPE RESULTS WORKSHEETS

Strontium

Raw, uncorrected concentrations (in ng) of Sr were derived from mass spectrometer readings.

Corrected concentration equation:

$$\textbf{Sr (ppm)} = \frac{\text{(init dilution/dilution used)(column dissolution/vol loaded)(raw ng)}}{\text{(powder wt*1000)}}$$

$$\text{ex. } \textbf{CIL-001} = \frac{(3/2)(200/100)(1372)}{(0.0507*1000)} = \textbf{81.20 ppm}$$

Table F-1. Semi-quantitative Sr concentration calculation matrix.

	powder weight (g)	initial dilution (mL)	dilution used (mL)	Column dissolved (µL)	loaded (µL)	Sr (ng)	Sr (ppm)
CIL-001	0.0507	3	2	200	100	1372	81
CIL-003	0.0867	3	2	200	100	593	21
CIL-004	0.0564	3	2	200	100	555	30
CIL-005	0.0984	3	2	200	100	2722	83
CIL-007	0.1168	3	2	200	100	4773	123
CIL-009	0.0829	3	2	350	100	1638	104
CIL-011	0.1278	3	2	200	100	—	—
CIL-012	0.1318	3	2	200	100	1420	32
CIL-013	0.0948	3	2	350	100	204	11
CIL-015	0.0753	3	2	350	100	1131	79
CIL-020	0.1656	3	2	200	100	3580	65
CIL-021	0.0906	3	2	350	100	1535	89
CIL-025	0.1171	3	2	200	100	3300	85
CIL-026	0.0189	3	2	350	100	1755	488
CIL-028	0.0175	3	2	350	100	819	246
CIL-029	0.0563	3	2	350	100	1560	145
CIL-032	0.0367	3	2	350	100	1560	223
CIL-033	0.0487	3	2	350	100	1120	121
CIL-034	0.0254	3	2	350	100	1365	282
CIL-037	0.1061	3	2	200	100	1772	50
CIL-039	0.0973	3	2	350	100	3300	178
CIL-040	0.1583	3	2	200	100	1852	35
CIL-042	0.1042	3	2	200	100	5444	157
CIL-043	0.1581	3	2	200	100	2722	52
CIL-044	0.0755	3	2	200	100	332	13
CIL-046	0.1089	3	2	200	100	2600	72
CIL-047	0.1403	3	2	200	100	4171	89
CIL-048	0.0427	3	2	200	100	1600	112
CIL-049	0.1019	3	2	200	100	1155	34
CIL-050	0.1400	3	2	200	100	6333	136
CIL-052	0.1241	3	2	200	100	1914	46
CIL-054	0.1030	3	2	200	100	930	27
CIL-055	0.1137	3	2	200	100	1400	37
CIL-058	0.0640	3	2	200	100	1444	68
CIL-059	0.1432	3	2	350	100	2730	100
CIL-060	0.1365	3	2	200	100	3333	73

Table F-1. Continued.

	powder weight (g)	initial dilution (mL)	dilution used (mL)	Column dissolved (µL)	loaded (µL)	Sr (ng)	Sr (ppm)
AFA-003	0.1007	3	2	350	100	1949	102
AFA-004	0.1011	3	2	350	100	1999	104
AFA-006	0.1252	3	2	200	100	940	23
AFA-017	0.1244	3	2	200	100	270	7
AFA-021	0.1328	3	2	200	100	1432	32
AFA-023	0.1436	3	2	350	100	1016	37
AFA-025	0.1429	3	2	350	100	776	29
AFA-031	0.1267	3	2	200	100	1016	24
AFA-032	0.1046	3	2	350	100	707	35
AFA-047	0.1106	3	2	350	100	742	35
AFA-051	0.1129	3	2	200	100	785	21
AFA-056	0.1039	3	2	200	100	587	17
AFA-060	0.1030	3	2	350	100	942	48
AFA-063	0.0650	3	2	350	100	977	79
AFA-075	0.1111	3	2	350	100	1094	52
AFA-078	0.1000	3	2	350	100	1691	89
AFA-085	0.1060	3	2	200	100	559	16
AFA-086	0.1120	3	2	200	100	1316	35
AFA-089	0.1090	3	2	200	100	740	20
AFA-096	0.1090	3	2	350	100	667	32
AFA-103	0.1020	3	2	200	100	390	11
AFA-109	0.1020	3	2	350	100	993	51
AFA-111	0.1070	3	2	200	100	243	7
AFA-116	0.1212	3	2	200	100	1298	32
AFA-133	0.0977	3	2	200	100	404	12
AFA-134	0.1327	3	2	350	100	554	22
AFA-143	0.1343	3	2	350	100	533	21
AFA-146	0.0988	3	2	200	100	559	17
AFA-148	0.1063	3	2	350	100	1287	64
AFA-163	0.0912	3	2	350	100	726	42
AFA-164	0.1152	3	2	200	100	137	4
AFA-173	0.0887	3	2	200	100	252	9
AFA-174	0.0945	3	2	200	100	587	19
AFA-176	0.1578	3	2	200	100	705	13
AFA-184	0.0683	3	2	350	100	746	57
AFA-220	0.0949	3	2	350	100	1211	67

Lead

Raw, uncorrected concentrations (in ng) of Pb were derived from mass spectrometer readings.

Corrected concentration equation:

$$\textbf{Pb (ppm)} = \frac{\text{(init dilution/dilution used)(column dissolution/vol loaded)(raw ng)}}{\text{(powder wt*1000)}}$$

$$\text{ex.}\quad \textbf{CIL-001} = \frac{(3/2)(300/200)(27)}{(0.0507*1000)} = \textbf{1.20 ppm}$$

Table F-2. Semi-quantitative Sr concentration calculation matrix.

	powder weight (g)	initial dilution (mL)	dilution used (mL)	Column dissolved (μL)	loaded (μL)	Pb (ng)	Pb (ppm)
CIL-001	0.0507	3	2	300	200	27	1.2
CIL-003	0.0867	3	2	600	200	211	11.0
CIL-004	0.0564	3	2	300	200	106	4.2
CIL-005	0.0984	3	2	300	200	290	6.6
CIL-007	0.1168	3	2	300	200	240	4.6
CIL-009	0.0829	3	2	600	200	70	3.8
CIL-011	0.1278	3	2	300	200	60	1.1
CIL-012	0.1318	3	2	300	200	250	4.3
CIL-013	0.0948	3	2	600	200	550	26.1
CIL-015	0.0753	3	2	600	200	120	7.2
CIL-020	0.1656	3	2	300	200	28	0.4
CIL-021	0.0906	3	2	600	200	310	15.4
CIL-025	0.1171	3	2	300	200	40	0.8
CIL-026	0.0189	3	2	600	200	125	29.8
CIL-028	0.0175	3	2	600	200	600	154.3
CIL-029	0.0563	3	2	600	200	190	15.2
CIL-032	0.0367	3	2	600	200	350	42.9
CIL-033	0.0487	3	2	600	200	42	3.9
CIL-034	0.0254	3	2	600	200	1400	248
CIL-037	0.1061	3	2	300	200	70	1.5
CIL-039	0.0973	3	2	600	200	350	16.2
CIL-040	0.1583	3	2	300	200	48	0.7
CIL-042	0.1042	3	2	300	200	80	1.7
CIL-043	0.1581	3	2	300	200	20	0.3
CIL-044	0.0755	3	2	600	200	159	9.5
CIL-046	0.1089	3	2	300	200	327	6.8
CIL-047	0.1403	3	2	600	200	101	3.2
CIL-048	0.0427	3	2	300	200	180	9.5
CIL-049	0.1019	3	2	600	200	326	14.4
CIL-050	0.1400	3	2	300	200	960	15.4
CIL-052	0.1241	3	2	300	200	230	4.2
CIL-054	0.1030	3	2	600	200	120	5.2
CIL-055	0.1137	3	2	600	200	507	20.1
CIL-058	0.0640	3	2	300	200	207	7.3
CIL-059	0.1432	3	2	600	200	150	4.7
CIL-060	0.1365	3	2	300	200	105	1.7

Table F-2. Continued.

	powder weight (g)	initial dilution (mL)	dilution used (mL)	Column dissolved (µL)	Column loaded (µL)	Pb (ng)	Pb (ppm)
AFA-003	0.1007	3	2	400	200	4.6	0.14
AFA-004	0.1011	3	2	400	200	2.0	0.06
AFA-006	0.1252	3	2	600	200	4.0	0.14
AFA-017	0.1244	3	2	600	200	2.6	0.09
AFA-021	0.1328	3	2	600	200	3.3	0.11
AFA-023	0.1436	3	2	400	200	1.9	0.04
AFA-025	0.1429	3	2	400	200	19.1	0.40
AFA-031	0.1267	3	2	600	200	12.2	0.43
AFA-032	0.1046	3	2	400	200	5.3	0.15
AFA-047	0.1106	3	2	400	200	25.2	0.68
AFA-051	0.1129	3	2	600	200	3.6	0.14
AFA-056	0.1039	3	1*	400	200	0.3	0.02
AFA-060	0.1030	3	2	400	200	2.8	0.08
AFA-063	0.0650	3	2	400	200	4.2	0.19
AFA-075	0.1111	3	2	400	200	5.2	0.14
AFA-078	0.1000	3	2	400	200	3.1	0.09
AFA-085	0.1060	3	2	600	200	4.1	0.17
AFA-086	0.1120	3	2	600	200	6.5	0.26
AFA-089	0.1090	3	2	600	200	5.9	0.24
AFA-096	0.1090	3	2	400	200	3.9	0.11
AFA-103	0.1020	3	2	600	200	7.3	0.32
AFA-109	0.1020	3	2	400	200	17.0	0.50
AFA-111	0.1070	3	2	600	200	2.6	0.11
AFA-116	0.1212	3	2	600	200	25.5	0.95
AFA-133	0.0977	3	2	600	200	1.1	0.05
AFA-134	0.1327	3	2	400	200	2.1	0.05
AFA-143	0.1343	3	2	400	200	15.8	0.35
AFA-146	0.0988	3	2	600	200	1.4	0.06
AFA-148	0.1063	3	2	400	200	21.4	0.60
AFA-163	0.0912	3	2	400	200	3.6	0.12
AFA-164	0.1152	3	2	600	200	2.4	0.09
AFA-173	0.0887	3	2	600	200	2.8	0.14
AFA-174	0.0945	3	2	600	200	4.3	0.20
AFA-176	0.1578	3	2	600	200	3.0	0.09
AFA-184	0.0683	3	2	400	200	59.1	2.60
AFA-220	0.0949	3	2	400	200	29.0	0.92

*Note: AFA-056 had to be rerun through column, so back-up dilution used.

Table F-3. Comparison of the means for multiple runs of the GLM procedure for Pb. (CIL outlier excluded)

Group	Variable	N	Mean	Std Dev	Min	Max
East Asia	$^{208}Pb/^{204}Pb$	35	38.074157	0.355069	37.17610	38.88370
	$^{207}Pb/^{204}Pb$	35	15.603740	0.040095	15.52880	15.69580
	$^{206}Pb/^{204}Pb$	35	18.090809	0.435832	16.99170	19.62050
	$^{208}Pb/^{206}Pb$	35	2.105517	0.040400	1.95801	2.25445
	$^{207}Pb/^{204}Pb$	35	0.862969	0.018726	0.79998	0.91475
USAFA	$^{208}Pb/^{204}Pb$	36	38.268931	0.225698	37.39830	38.61650
	$^{207}Pb/^{204}Pb$	36	15.631292	0.022565	15.55680	15.67490
	$^{206}Pb/^{204}Pb$	36	18.595094	0.280822	17.68100	19.04930
	$^{208}Pb/^{206}Pb$	36	2.058318	0.020901	2.02726	2.11503
	$^{207}Pb/^{204}Pb$	36	0.840787	0.011796	0.82286	0.87981
USAFA (US only)	$^{208}Pb/^{204}Pb$	30	38.318790	0.121833	37.98040	38.61650
	$^{207}Pb/^{204}Pb$	30	15.637293	0.015895	15.59830	15.67490
	$^{206}Pb/^{204}Pb$	30	18.683780	0.168947	18.23380	19.04930
	$^{208}Pb/^{206}Pb$	30	2.051028	0.012452	2.02726	2.08576
	$^{207}Pb/^{204}Pb$	30	0.837004	0.006806	0.82286	0.85565
USAFA (foreign)	$^{208}Pb/^{204}Pb$	6	38.019633	0.425429	37.39830	38.55000
	$^{207}Pb/^{204}Pb$	6	15.601283	0.028323	15.55680	15.63150
	$^{206}Pb/^{204}Pb$	6	18.151667	0.321455	17.68100	18.47340
	$^{208}Pb/^{206}Pb$	6	2.094765	0.015675	2.07610	2.11503
	$^{207}Pb/^{204}Pb$	6	0.859700	0.013795	0.84619	0.87981
USAFA (CONUS)	$^{208}Pb/^{204}Pb$	27	38.337078	0.106481	38.02970	38.61650
	$^{207}Pb/^{204}Pb$	27	15.639315	0.014631	15.60130	15.67490
	$^{206}Pb/^{204}Pb$	27	18.705548	0.155096	18.23380	19.04930
	$^{208}Pb/^{206}Pb$	27	2.049609	0.011729	2.02726	2.08576
	$^{207}Pb/^{204}Pb$	27	0.836129	0.006243	0.82286	0.85565
USAFA (overseas)	$^{208}Pb/^{204}Pb$	9	38.064489	0.351164	37.39830	38.55000
	$^{207}Pb/^{204}Pb$	9	15.607222	0.025748	15.55680	15.63160
	$^{206}Pb/^{204}Pb$	9	18.263733	0.319593	17.68100	18.63290
	$^{208}Pb/^{206}Pb$	9	2.084446	0.020978	2.05207	2.11503
	$^{207}Pb/^{204}Pb$	9	0.854759	0.013758	0.83892	0.87981

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